EXHIBIT 12

Incidence of health insurance claims for thyroid neoplasm and pancreatic malignancy in association with exenatide: signal refinement using active safety surveillance

David D Dore, John D Seeger and K Arnold Chan
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David D. Dore, John D. Seeger and K. Arnold Chan

Abstract:
Objectives: As part of a regulatory postmarketing commitment, we assessed the risk of claims for thyroid and pancreatic cancer among users of exenatide using an active drug safety surveillance system.
Methods: This active surveillance assessment used cohort methodology and commercial health insurance claims data to identify initiators of exenatide and propensity score-matched initiators of metformin or glyburide between June 2005 and September 2009, with up to 1 year of follow up through December 2009. The primary analysis estimated absolute and relative risk (RR) of inpatient or outpatient claims with diagnosis codes for thyroid neoplasm (benign or malignant) or pancreatic malignancies after exclusion of patients with a history of the same diagnosis at baseline.
Results: Among the matched comparison cohorts ($N \approx 32{,}800$ each), there were 37 claims-suggested thyroid malignancies among exenatide initiators and 26 among metformin or glyburide initiators [RR 1.4; 95% confidence interval (CI) 0.8–2.4]. This association was attenuated when limited to inpatient thyroid cancer claims (RR 0.9; CI 0.3–2.6). Exenatide use was not associated with an increased risk of benign thyroid neoplasm (RR 0.7; CI 0.3–1.7), or pancreatic cancer (RR 0.8; CI 0.5–1.6).
Conclusions: Use of exenatide was associated with a modestly higher incidence of inpatient and outpatient claims, but not inpatient claims for thyroid malignancies. Exenatide was not associated with higher risk of benign thyroid neoplasm or pancreatic cancer. Misclassification of outcomes and exposure, and residual confounding remain limitations of this analysis to be considered when interpreting the results. We have initiated a formal epidemiologic investigation to explore these relationships.

Keywords: active safety surveillance, exenatide, pancreatic cancer, safety signal, thyroid cancer

Correspondence to:
David D. Dore, PharmD, PhD
Brown University, Box G-121-7, 121 South Main Street, Providence, RI 02912, USA
david_dore@brown.edu
John D. Seeger, PharmD, ScD
OptumInsight Epidemiology, Waltham, MA; Division of Pharmacoepidemiology and Pharmacoeconomics, Harvard Medical School/ Brigham and Women's Hospital, Boston, MA, USA; Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA
K. Arnold Chan, MD, ScD
OptumInsight Epidemiology, Waltham, MA; Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA

Introduction

Exenatide is an incretin mimetic that when taken twice daily enhances endogenous insulin production, suppresses postprandial glucagon, and reduces food intake [Byetta Prescribing Information, 2010]. Another incretin mimetic, liraglutide, was found to increase the risk of c-cell tumors in rodents exposed to clinically relevant doses, leading to concern about the occurrence of medullary thyroid cancer (MTC), a c-cell cancer, in humans [Victoza Prescribing Information, 2010]. In January 2010, the US Food and Drug Administration (FDA) approved liraglutide. The product's approval was contingent on the manufacturer conducting or sponsoring a number of post-marketing studies, including studies to assess the association between liraglutide and MTC, and thyroid cancer generally [Parks and Rosebraugh, 2010].

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Following the rodent findings of c-cell cancers associated with liraglutide, concerns have been raised about thyroid cancer outcomes associated with all incretin mimetics. Concerns also exist about the risk of pancreatic cancer secondary to incretin mimetics [Elashoff *et al.* 2011]. In October 2009, the FDA approved a new indication for exenatide as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus, including monotherapy with exenatide. As part of the approval, the FDA requested that the manufacturer of exenatide conduct an assessment of the thyroid and pancreatic cancer signals using an active safety surveillance system. We report data from this active safety surveillance system (i3 Aperio, OptumInsight Epidemiology, Waltham, MA, USA) on the incidence of health insurance claims for thyroid neoplasm (benign or malignant separately and together) and pancreatic cancer in people who initiated exenatide relative to a propensity score-matched cohort that initiated metformin or glyburide, as a safety signal refinement exercise.

Patients and methods

Data source

The methods and data source of the safety surveillance system with respect to exenatide are published elsewhere [Dore *et al.* 2009]. The source population came from the Normative Health Information (NHI) database, a large, geographically diverse population of health insurance plan enrollees. The records in the NHI database include provider and facility claims, outpatient pharmacy dispensing records, and an enrollment file that contains demographic data and dates of insurance eligibility for people on the database.

Formation of comparison groups

The analysis included patients who initiated the twice-daily formulation of exenatide or metformin or glyburide and were listed on the NHI database between 1 June 2005 and 30 September 2009, with follow up through 31 December 2009. Initiation was defined as a dispensing of the study drug preceded by 6 months of continuous health plan enrollment without a dispensing of the same drug. Exposure status during follow up (exenatide or metformin/glyburide) was defined as the drug dispensed that qualified the patient for cohort entry. Patients in the exenatide cohort were matched to those in the metformin or glyburide cohort on the propensity score [Seeger *et al.* 2005]. The baseline covariates were ascertained from the 6 months of claims data preceding the date of study drug initiation.

The propensity score analysis involved two stages, the first being the development of the propensity score using baseline patient characteristics. These variables were determined from the NHI database from the time leading up to patients' entry into the cohorts. The second stage involved matching exenatide initiators to initiators of metformin or glyburide using a greedy matching algorithm that first identified patients with matching propensity scores to eight decimal digits of precision and was iteratively loosened by one decimal digit, stopping at the first decimal digit. Matching on propensity scores results in two study cohorts with similar prevalence of characteristics that are included in the model at the start of treatment [Rosenbaum and Rubin, 1983].

The final propensity score model included variables representing age, sex, geographic region, paid hospital costs, paid pharmacy costs, paid emergency room costs, total paid insurance costs, total paid patient costs, and the number of unique three-digit International Classification of Disease (ICD) diagnoses, drugs, physician visits, emergency room visits, hospital stay days, laboratory tests, procedures, days available for the baseline period, and total days of enrollment.

Outcomes and analysis

Follow up occurred from cohort entry until 1 year following initiation of the study drug or disenrollment from the health insurance plan, whichever was earliest. We tabulated the prevalence of baseline characteristics derived from insurance claims in the 6 months before cohort entry. The exposure classification was an analog of intention-to-treat analysis. Each day of follow up, the patient was considered to be exposed to the baseline exposure category (exenatide *versus* metformin or glyburide) and subsequent changes in the medication regimen were ignored. We estimated the cumulative incidence of thyroid neoplasm or pancreatic cancer, the relative risk (RR) across cohorts, and 95% confidence intervals (CIs). The outcomes were identified by the presence of one or more inpatient or outpatient claims during follow up associated with pancreatic cancer [ICD, 9th revision (ICD-9) 157.xx], benign thyroid neoplasm (ICD-9 226), or malignant thyroid neoplasm (ICD-9 193). In the primary analysis, we limited estimation of the absolute and relative risk (using 2 × 2 tables) to patients who had no claim for the same diagnosis in the 6-month baseline period (treatment-emergent outcomes).

This assessment included three sensitivity analyses. The first analysis included a lag period between cohort entry and when follow-up person-time was considered at risk, an approach aimed at mitigating the potential attenuation of the RR that can result when patients are considered at risk for the outcomes immediately after the initiation of exposure, but when the outcomes are expected to occur after some induction or latency period. We excluded from the numerator of the risk calculations cases that occurred in the first 90 or 180 days, separately, using the primary (inpatient and outpatient) outcome definition. Second, we restricted identification of the outcomes to inpatient facility claims with the code of interest listed in the first position with the aim of understanding whether this approach might be less biased than the primary approach of also including outpatient physician claims for outcome identification. The third sensitivity analysis aimed to remove remaining imbalance in the utilization of healthcare services across the exposure cohorts through a stratified analysis. We estimated the RR of thyroid cancer based on the primary (inpatient and outpatient) outcome definition within strata defined by the number physician visits (1–3, 4–6, or ≥7) in the 6 months prior to cohort entry. The latter two sensitivity analyses were among all patients (before exclusion of prevalent cases) after the observation that exclusion according to cancer history did not appreciably alter the RR estimates.

Results

Table 1 lists select baseline characteristics of patients in the exenatide and metformin or glyburide cohorts. There were 32,894 patients in each matched cohort prior to exclusion for baseline history of the cancers of interest. A small number of patients were excluded from each cohort upon estimation of cancer incidence proportions (Table 2). The cohorts had similar age and sex distributions, with about two-thirds of the population aged between 40 and 59 years, and approximately 55% women. There were residual imbalances in a number of baseline patient characteristics, including a higher baseline prevalence of a recorded diabetes diagnosis, retinal disorders, use of lipotropics, and use of several antihyperglycemic drugs in the exenatide cohort.

The median days of drug supply received by the exenatide cohort was 140 days across a median of four dispensings (Table 2). The median time between first and last exenatide dispensing was 234 days and 33.9% of patients in the exenatide cohort received a dispensing of that drug within 30 days of the end of follow up, indicating ongoing or continued use.

The absolute risk of claims for all study outcomes was as high as 0.4% in the follow up of the overall cohorts (≤1 year), but was reduced to less than 0.2% after baseline exclusions for the same cancer (Table 3). After these exclusions, there were 46 patients with claims-suggested thyroid neoplasm among exenatide initiators and 40 among metformin or glyburide initiators (RR 1.2; 95% CI 0.7–1.8). The estimated risk of claims for benign thyroid neoplasms was similar across the two cohorts (RR 0.7; 95% CI 0.3–1.7), while we observed a somewhat higher risk of claims for thyroid malignancies (RR 1.4; 95% CI 0.8–2.4) in the exenatide cohort. The observed incidence of claims for pancreatic cancer was similar in the exenatide cohort relative to comparators (RR 0.8; 95% CI 0.5–1.6). The RR estimates from the cohorts before baseline exclusions were similar to the treatment-emergent values.

The results of the sensitivity analyses were generally consistent with the overall results. Restriction to outcomes identified from first-position diagnosis codes on inpatient claims resulted in a reduced RR for pancreatic and thyroid outcomes, but wider confidence intervals. For thyroid malignancy, the estimated RR was 0.9 (95% CI 0.3–2.6). The estimated RRs without cases from the first 90 or 180 days of follow up were similar to the main results. The RRs within the strata of one to three and four to six baseline physician visits were also similar to the overall results; however, the RR of thyroid cancer was attenuated among patients with at least seven baseline physician visits.

Discussion

We found that exenatide use was associated with a somewhat higher incidence of combined outpatient and inpatient health insurance claims, but no increased incidence of inpatient claims for

Table 1. Select baseline demographic and clinical characteristics of exenatide and metformin or glyburide initiators in the Normative Health Information database after propensity-score matching, 1 June 2005–30 September 2009.*

	Exenatide initiators (*N* = 32,894)		Metformin or glyburide initiators (*N* = 32,894)	
	N	%	*N*	%
Demographic characteristics				
Age				
≤ 19	93	0.3	100	0.3
20–39	3697	11.2	3528	10.7
40–49	8106	24.6	8100	24.6
50–59	13,043	39.7	13,156	40.0
≥ 60	7955	24.2	8010	24.4
Women	18,033	54.8	18,314	55.7
Race				
African American/non-Hispanic black	1603	4.9	1780	5.4
Asian	217	0.7	363	1.1
Hispanic	1669	5.1	1712	5.2
Non-Hispanic white	17,594	53.5	16,615	50.5
Other or unknown race	11,811	35.9	12,424	37.8
Baseline diagnoses				
Diabetes mellitus (ICD-9 250)	26,673	81.1	16,195	49.2
Disorders of lipid metabolism (ICD-9 272)	18,357	55.8	13,726	41.7
Essential hypertension (ICD-9 401)	18,185	55.3	15,659	47.6
Overweight, obesity, or other hyperalimentation (ICD-9 278)	4296	13.1	2355	7.2
Cardiac dysrhythmias (ICD-9 427)	1042	3.2	1190	3.6
Heart failure (ICD-9 428)	777	2.4	807	2.5
Acquired hypothyroidism (ICD-9 244)	3031	9.2	2640	8.0
Other retinal disorders (ICD-9 362)	1124	3.4	494	1.5
Chronic kidney disease (ICD-9 585)	682	2.1	294	0.9
Top 10 pharmacy dispensing				
Hypoglycemics, biguanide type (non-sulfonylureas)	16,383	49.8	3	0.0
Lipotropics	16,287	49.5	12,961	39.4
Hypoglycemics, insulin-release stimulant type	14,746	44.8	4902	14.9
Blood sugar diagnostics	13,522	41.1	7797	23.7
Hypoglycemics, insulin-response enhancer (non-sulfonylureas)	13,357	40.6	5434	16.5
Hypotensive, angiotensin-converting enzyme inhibitors	11,993	36.5	9125	27.7
Needles/needleless devices	10,196	31.0	1074	3.3
Analgesics, narcotics	8291	25.2	11,193	34.0
Hypotensive, angiotensin receptor antagonist	7686	23.4	5986	18.2
Insulins	7700	23.4	3373	10.3
Healthcare utilization				
Total costs, US$ (mean, median)	4799	2551	4967	2224
Number of physician visits (mean, median)	5.2	4.0	5.3	4.0
Number of drugs dispensed	11.0	10.0	11.1	10.0

*Data derived from claims for healthcare services in the 6 months prior to study drug initiation using the i3 Aperio (OptumInsight Epidemiology, Waltham, MA, USA) active drug safety surveillance system.
ICD-9, International Classification of Disease.

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Table 2. Characteristics of utilization of exenatide and metformin/glyburide during follow up among exenatide and metformin or glyburide initiators, Normative Health Information database, 1 June 2005–31 December 2009.

	Exenatide initiators (*N* = 32,894)			Metformin or glyburide initiators (*N* = 32,894)		
	Mean	Median	IQR	Mean	Median	IQR
Exenatide use						
Number of people with at least one dispensing during follow up (*N*, %)	32,894	100.0		1,521	4.6	
Number of dispensings per person	4.8	4.0	5.0	3.7	3.0	4.0
Total days supplied per person	167.5	140.0	210.0	129.9	90.0	120.0
Drug strength (μg)	8.0	8.8	5.0	8.0	8.9	5.0
Time from first to last dispensing (days)	217.2	234.0	227.0	160.8	144.0	173.0
Medication possession ratio	0.8	0.8	0.4	0.9	0.8	0.4
Patients with dispensing within 30 days before end of follow up (*N*, %)	11,155	33.9		646	2.0	
Metformin or glyburide use						
Number of people with at least one dispensing during follow up (*N*, %)	20,101	61.1		32,894	100.0	
Number of dispensings per person	6.0	5.0	6.0	5.3	4.0	6.0
Total units dispensed per person (tablets)	627.3	540.0	570.0	382.3	300.0	450.0
Total days supplied per person	231.0	240.0	210.0	190.4	180.0	240.0
Quantity per day (tablets)	2.7	2.0	2.0	2.0	2.0	0.5
Time from first to last dispensing (days)	238.7	271.0	168.0	235.9	277.0	213.0
Medication possession ratio	0.9	0.9	0.3	0.8	0.9	0.4
Patients with dispensing within 30 days before end of follow up (*N*, %)	10,990	33.4		13,977	42.5	

IQR, interquartile range.

thyroid malignancy. Exenatide use was not associated with increased incidence of claims for benign thyroid neoplasm or pancreatic cancer compared with glyburide/metformin use. The surveillance system used for this evaluation is a signal generation and refinement tool that allowed for rapid (within 1 week of learning of the initial signal) assessment of a safety signal of these rare neoplasms in association with exenatide adding to information from clinical trials and spontaneous adverse drug reaction reports. The features of clinical trials that promote valid and efficient assessments of efficacy represent limitations in the context of safety surveillance. Their generally small size, homogeneous populations, and short-term follow up means that adverse outcomes occurring in less than 1 in 1000 patients tend not to be reliably identified and investigated [ICH, 1995], and this limitation cannot be addressed in the context of the premarket assessment without adding considerably to the time and expense of drug approval [Committee on the Assessment of the US Drug Safety System, 2007]. Active safety surveillance systems provide context to safety signals derived from spontaneous reports by allowing for a rapid assessment of signals in a population with a known denominator, allowing for estimation of incidence, and in the case of this analysis, control for some differences in baseline risk for the outcomes across the exposure cohorts through propensity-score matching.

However, the surveillance system and the source data have limitations that warrant discussion [Crystal *et al.* 2007; Walker, 2001]. Health insurance claims data are collected for the purpose of justifying and tracking reimbursement to providers and facilities for healthcare services rendered, and include certain descriptions of the patients and services performed for those

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Table 3. Absolute and relative risk of treatment-emergent inpatient and outpatient claims associated with diagnoses of pancreatic and thyroid neoplasm among exenatide and metformin or glyburide initiators, Normative Health Information database, 1 June 2005–31 December 2009.

	Cases (*N*)	Patients (*N*)	Absolute risk (%)	Relative risk	95% CI
Cases identified from inpatient or outpatient claims					
All thyroid neoplasms					
Exenatide	46	32,807	0.1	1.2	0.7–1.8
Metformin/glyburide	40	32,828	0.1	1	reference
Benign thyroid neoplasms					
Exenatide	11	32,877	0	0.7	0.3–1.7
Metformin/glyburide	15	32,879	0	1	reference
Thyroid malignancies					
Exenatide	37	32,822	0.1	1.4	0.8–2.4
Metformin/glyburide	26	32,842	0.1	1	reference
Pancreatic malignancy					
Exenatide	21	32,889	0.1	0.8	0.5–1.6
Metformin/glyburide	25	32,878	0.1	1	reference
Cases identified from inpatient claims only					
All thyroid neoplasms					
Exenatide	7	32,894	0.0	0.7	0.2–2.0
Metformin/glyburide	10	32,894	0.0	1.0	reference
Benign thyroid neoplasms					
Exenatide	0	32,894	0.0	0.0	0.0–4.1
Metformin/glyburide	2	32,894	0.0	1.0	reference
Thyroid malignancies					
Exenatide	7	32,894	0.0	0.9	0.3–2.6
Metformin/glyburide	8	32,894	0.0	1.0	reference
Pancreatic malignancy					
Exenatide	12	32,894	0.0	0.5	0.2–1.1
Metformin/glyburide	23	32,894	0.1	1.0	reference

CI, confidence interval.

purposes. Because these descriptors are not collected for clinical care or research purposes, lack of correspondence between this information and true patient disposition can result in biased RRs [Lanes and de Luise, 2006]. Of particular relevance here is the correspondence between the diagnosis codes for the neoplasm outcomes and the patient's actual diagnosis (or lack thereof). Others have shown that assessments of cancer outcomes in health insurance claims data that define the cancer based on a single diagnosis code can be problematic because the outcome definition will have a low positive predictive value and misclassify some patients' cancer status [Setoguchi *et al.* 2007]. Indeed, the incidence estimates from our primary (inpatient and outpatient) data are substantially higher than the 5.2–15.2 cases per 100,000 person-years observed in population-based cancer surveillance, consistent with inclusion of false-positive cases from the health insurance claims; however, the diagnoses from first-position inpatient claims resulted in more plausible incidence estimates [Altekruse *et al.* 2010]. The analyses that did not exclude patients with claims for the cancer outcomes in the baseline period resulted in even higher incidence estimates relative to the treatment-emergent analysis, reflecting the identification of prevalent cases during follow up.

In studies based on health insurance claims data, this type of error resulting from the outcome definition will generally bias the RRs toward showing no effect – although this direction of bias need not be the case [Jurek *et al.* 2008]. A form of surveillance bias when thyroid cancer was more readily detected among exenatide users is one potential explanation for the finding of excess risk

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of thyroid cancer claims in the exenatide cohort. This differential detection could plausibly occur if exenatide users sought more healthcare services during follow up, a difference that was evident in a previous study of exenatide [Dore *et al.* 2011]. We aimed to address this potential surveillance bias by stratifying the RR estimation by the number of physician visits observed in the baseline period among all patients (before exclusion) with the rationale being that within these strata, overall healthcare utilization might be similar during follow up.

Another consideration is that if the relationship between exenatide and these outcomes (should it exist) has a long induction or latency period, then any increase in the risk of the outcomes due to exenatide exposure would not be observed in this study, in which the average follow-up time was less than 1 year. In the absence of other biases, the estimated RR would be attenuated with insufficient follow up if there was a true effect of exenatide on thyroid cancer. With the outcome of thyroid malignancy in this study, in which the RR was 1.4, this type of bias did not appear to be sufficiently strong to obscure the signal altogether, but may still have biased the estimate toward showing no effect; or alternatively, any bias through this mechanism was negligible relative to other sources of bias (e.g. residual confounding).

Insufficient follow-up time might have resulted in a more severely biased RR estimate in a previous assessment of exenatide and thyroid cancer we conducted in the active safety surveillance system. In this previous assessment, cohort follow up was censored upon the apparent discontinuation of the study drug (exenatide or metformin/glyburide), reducing the average length of follow up. With this methodology, the analysis was consistent with no association between exenatide use and thyroid malignancy using the same outcome definitions and propensity score technique (data not shown).

It is also possible that the surveillance system's characterization of exposure to exenatide and metformin or glyburide affected the study results. First, this type of analysis assumes that pharmacy dispensings for the study drugs reflect patient consumption. While it is likely that some patients who received the study drugs did not take them as prescribed, these data are generally accepted as accurate [Crystal *et al.* 2007; McKenzie *et al.* 2000], and are at least as accurate as patient report [Leister *et al.* 1981; West *et al.* 1995]. Despite the probable accuracy of the pharmacy claims data, it remains possible that the average duration of exposure to exenatide in this assessment was insufficient to affect the incidence of thyroid malignancy and pancreatic cancer. To our knowledge, there are no data to inform whether the median apparent duration of exenatide exposure in this study (140 days) was sufficient to induce the malignancies of interest. Additionally, this analysis did not account for switching off study drugs; rather all patients were categorized as exposed from the time of cohort entry until the end of his or her follow up, although this exposure categorization may be appropriate for cancer outcomes.

The propensity-score matching applied by the active safety surveillance system removed many baseline differences in potential risk factors for the outcomes between the two exposure cohorts. Indeed, in the case of rare outcomes among large cohorts, propensity scores perform particularly well because they can account for many variables that might be associated with a higher risk of the outcomes among one of the exposure cohorts [Seeger *et al.* 2005]. However, residual differences in the baseline risk can remain if the propensity score does not include measures for all of the relevant predictors of the outcome, as might be the case with the parsimonious model the surveillance system employed for the present comparison [Rosenbaum and Rubin, 1983]. Depending on the association between these variables not included in the propensity score and the exposure and outcome, the observed RRs can be spuriously higher or lower as a result. This limitation of the present analysis is a reasonable alternative explanation for the observation of a higher risk of claims for thyroid malignancy in the exenatide cohort. We observed a higher prevalence of a number of indicators of diabetes severity in the exenatide cohort, and if diabetes severity or its treatment results in thyroid cancer, then residual confounding would potentially explain the observed results [e.g. Currie *et al.* 2009].

In summary, we observed a marginally higher incidence of combined outpatient and inpatient claims for thyroid malignancy, but no increased risk of inpatient claims only for thyroid malignancies. No increased risk of benign thyroid neoplasm or pancreatic cancer in association with exenatide use was observed in this rapid safety assessment program. These findings should be considered in

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the context of the limitations outlined above, taken together, and greater clarity with respect to the long-term effect of exenatide will require further study so that appropriate benefit–risk evaluations can be made for the prescribing of exenatide. A formal epidemiologic study of exenatide use and thyroid cancer to address the limitations of this active safety assessment has been initiated.

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Conflict of interest statement

Drs Dore, Seeger, and Chan were employees of OptumInsight Epidemiology at the time this work was conducted. The research contract granted OptumInsight Epidemiology oversight of the study conduct, reporting, and interpretation, as well as final wording of any resulting manuscripts.

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CLINICAL—PANCREAS

Pancreatitis, Pancreatic, and Thyroid Cancer With Glucagon-Like Peptide-1–Based Therapies

MICHAEL ELASHOFF, ALEKSEY V. MATVEYENKO, BELINDA GIER, ROBERT ELASHOFF, and PETER C. BUTLER

Larry L. Hillblom Islet Research Center at David Geffen School of Medicine and Department of Biomathematics, University of California, Los Angeles, California

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BACKGROUND & AIMS: Glucagon-like peptide-1–based therapy is gaining widespread use for type 2 diabetes, although there are concerns about risks for pancreatitis and pancreatic and thyroid cancers. There are also concerns that dipeptidyl peptidase-4 inhibitors could cause cancer, given their effects on immune function. **METHODS:** We examined the US Food and Drug Administration's database of reported adverse events for those associated with the dipeptidyl peptidase–4 inhibitor sitagliptin and the glucagon-like peptide-1 mimetic exenatide, from 2004–2009; data on adverse events associated with 4 other medications were compared as controls. The primary outcomes measures were rates of reported pancreatitis, pancreatic and thyroid cancer, and all cancers associated with sitagliptin or exenatide, compared with other therapies. **RESULTS:** Use of sitagliptin or exenatide increased the odds ratio for reported pancreatitis 6-fold as compared with other therapies ($P < 2 \times 10^{-16}$). Pancreatic cancer was more commonly reported among patients who took sitagliptin or exenatide as compared with other therapies ($P < .008$, $P < 9 \times 10^{-5}$). All other cancers occurred similarly among patients who took sitagliptin compared with other therapies ($P = .20$). **CONCLUSIONS: These data are consistent with case reports and animal studies indicating an increased risk for pancreatitis with glucagon-like peptide-1–based therapy. The findings also raise caution about the potential long-term actions of these drugs to promote pancreatic cancer.**

Keywords: Side Effect; Toxicity; Tumor; Pancreas.

Hyperglycemia in type 2 diabetes is due to inadequate insulin secretion in the setting of insulin resistance. A new class of drugs has been introduced for treatment of type 2 diabetes that takes advantage of the properties of the gut hormone glucagon-like peptide-1 (GLP-1).[1] GLP-1 is secreted by L-type endocrine cells in the distal ileum in response to food ingestion and amplifies glucose-mediated insulin secretion.[2]

GLP-1 has a short half-life, degraded by the enzyme dipeptidyl peptidase-4 (DPP-4) in the circulation.[3] To accomplish sustained GLP-1 receptor activation therapeutically, 2 strategies have been developed. In one, GLP-1 agonists that are resistant to DPP-4 degradation are administered by injection, including exenatide (Byetta; Amylin Pharmaceuticals, San Diego, CA) and liraglutide (Victoza; Novo Nordisk, Bagsværd, Denmark).[4,5] The alternative strategy is use of inhibitors of DPP-4, such as sitagliptin (Januvia; Merck & Co, Inc, Whitehouse Station, NJ), when administered orally enhance levels of endogenously secreted GLP-1.[4,5]

The attributes of GLP-1–based therapy for type 2 diabetes have been extensively reviewed.[1,4–6] Interest has recently been focused on the potential adverse effects of these new therapies.[7,8] Nausea is relatively common with the injected GLP-1 receptor agonists. Acute pancreatitis after administration of exenatide was originally reported in the form of case reports,[9,10] but then followed by a cautionary letter from the US Food and Drug Administration (FDA).[11] Recently, a similar caution was made by the FDA with regard to pancreatitis associated with sitagliptin treatment.[12]

The manufacturers of exenatide and sitagliptin have suggested that the most likely reason for the apparent association between the use of these drugs and acute pancreatitis is the increased risk of pancreatitis in patients with type 2 diabetes.[13] Recent animal studies showing pancreatitis as a consequence of GLP-1 mimetic therapy challenge that assumption and raise concerns about whether the asymptomatic chronic pancreatitis might be an as yet undetected adverse effect of GLP-1–based treatment.[14,15] Moreover, because pancreatitis is a risk factor for pancreatic cancer, long-term GLP-1 receptor activation might lead to increased risk for pancreatic cancer.[16,17] It has also been suggested that immunomodulatory effects of DPP-4 inhibition might increase risk for all cancers.[18,19] Also, thyroid tumors were reported to be more common

Abbreviations used in this paper: AERS, adverse event reporting system; CI, confidence interval; DPP-4, dipeptidyl peptidase-4; FDA, Food and Drug Administration; GLP-1, glucagon-like peptide-1; OR, odds ratio.

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in rodent toxicology studies with the GLP-1 agonist liraglutide, although the relevance of this in humans has been questioned.[20]

Given the >20 million known patients with type 2 diabetes in the United States alone and the numerous GLP-1–based drugs either available now or in the final stages of development, the potential impact of adverse effects of this class of drugs is considerable. However, because this class of drugs is relatively newly available, there are limited data on adverse effects. In addition, available reports were sponsored by pharmaceutical companies and arguably have a limited capacity to detect adverse outcomes.[21,22] The purpose of the present study was to gain the best possible insight into these potential adverse effects by examining the FDA adverse event reporting system (AERS) database.

Materials and Methods

Study Design

The primary goal of this analysis was to use the FDA AERS database to assess the association between treatment with exenatide (Byetta) or sitagliptin (Januvia) and an adverse event report of pancreatitis, where the drugs were listed as the primary suspect associated with a pancreatitis report in the database. A secondary goal was to examine the FDA AERS database for reported pancreatic or thyroid cancer associated with use of exenatide or sitagliptin. Third, we used the FDA AERS database to examine reports of all cancers in association with use of sitagliptin and exentide. The FDA AERS database depends on spontaneous reporting and is subject to various reporting biases. For this reason, 2 levels of control were used for the analysis. First, 4 other diabetes medications, ie, rosiglitazone (Avandia; GlaxoSmithKline, London, UK), nateglinide (Starlix; Novartis, Basel, Switzerland), repaglinide (Prandin; Novo Nordisk, Bagsværd, Denmark), and glipizide, were selected as control drugs. Rosiglitazone has been reported to attenuate toxin-induced pancreatitis in rats[23] and to exacerbate pancreatic fat infiltration in high-fat–fed mice.[24] Rosiglitazone appears to be neutral with regard to cancer risk.[13] It has been suggested that sulfonylurea therapy might increase risk for pancreatitis[25] and solid tumors,[26] so these drugs should be a conservative choice as controls. Second, control events were prospectively defined that were believed a priori to have no association with either of the test drugs, exenatide/sitagliptin, or the control drugs.

The predefined events of interest were pancreatitis, pancreatic cancer, thyroid cancer, and all cancers. We prospectively defined 5 types of control events, including back pain, urinary tract infection, chest pain, cough, and syncope. By this approach, we were able to address the issue that pancreatitis[27] and pancreatic cancer[28] are more common in type 2 diabetes because test and control drugs are used for treatment of type 2 diabetes.

Database Inquiry. We downloaded the FDA AERS database for the period covering the first quarter of 2004 through third quarter of 2009. and applied the search terms listed below. As described in the study design, only primary suspect drugs were used in the analysis (ROLE_COD='PS'); cases with more than one primary suspect drug were counted for each drug. For pancreatitis, the search term "PANCREATITIS" was used. Control events used the search terms "BACK PAIN", "CHEST PAIN", "COUGH", "SYNCOPE", and "URINARY TRACT INFECTION". For pancreas cancer, the search terms "PANCREATIC MASS", "PANCREATIC NEOPLASM", "ADENOCARCINOMA PANCREAS" and "PANCREATIC CARCINOMA" were used. For thyroid cancer, the search terms "THYROID CANCER", "THYROID GLAND CANCER", "THYROID NEOPLASM" and "THYROID MASS" were used. For all other cancers, "THYROID" and "PANCREATIC" records were filtered out, and the search terms "LEUKAEMIA", "CANCER", "SARCOMA", "MYELO", "CARCINOM", "MALIGNAN", "NEOPLAS", "TUMOUR", "METASTASES", "MACROGLOBULINEMIA", "LYMPHOMA" , "MELANOMA", "BLASTOMA", "CYTOMA", "MENINGIOMA", "MESOTHELIOMA", "HODGKIN", "GLIOMA", "ADENOMA", "BLADDER MASS", "BRAIN MASS", "BREAST MASS", "HEPATIC MASS", "RENAL MASS", "INTESTINAL MASS", "LARYNGEAL MASS", "OESOPHAGEAL MASS", "OVARIAN MASS", "PHARYNGEAL MASS", "PROSTATIC MASS", "PULMONARY MASS", "UTERINE MASS", "TESTICULAR MASS", "STOMACH MASS", "SCROTAL MASS", "SALIVARY GLAND MASS", "ABDOMINAL MASS", "LYMPHADENO" and "RHABDOMYO" were used. For the analysis that used only events reported to have occurred prior to 2007, the same database was filtered by EVENT_DT<2007 prior to querying for the above terms (if EVENT_DT was missing, FDA_DT<2007 was used). For drugs, the following search terms were used: exenatide: "BYETTA", "EXENATIDE"; sitagliptin: "JANUVIA", "SITAGLIPTIN"; control drugs: "AVANDIA", "ROSIGLITAZONE", "STARLIX", "NATEGLINIDE", "PRANDIN", "REPAGLINIDE", "NOVONORM", "GLIPIZIDE" and "GLUCOTROL". In all cases, search terms were applied with a wildcard character before and after the search term.

Statistical Analysis

Two levels of control were used for the comparative analysis of event rates. The count of events of interest (eg, pancreatitis) in a test drug (eg, exenatide) were compared to control drugs and to control events (events for which there was the presumption of no drug–event relationship) using 2×2 tables. The premise on which the 2-level control is based is that under the null hypothesis of no elevated event rate for the test drugs, the odds ratio (OR) in the 2×2 table should be 1. Fisher's exact test was used to test the null hypothesis that the OR was equal to 1. Two-sided 95% confidence intervals were also constructed for the estimated ORs. The Breslow-Day test was used to test for homogeneity of odds-ratios by gender, and the Mantel-Haenszel test was used to perform gender stratified analyses. All statistical analyses were conducted

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Table 1. Test and Control Events for Exenatide and Sitagliptin vs Control Drugs

		PANCREATITIS		
Drug	Pancreatitis events	Control events	Odds ratio vs control drugs	*P*-value vs control drugs
Exenatide	971	1433	10.68	2×10^{-16}
Sitagliptin	131	306	6.74	2×10^{-16}
Controls	43	678	—	—
		PANCREATITIS (2006 AND PRIOR)		
Drug	Pancreatitis events	Control events	Odds ratio vs control drugs	*P*-value vs control drugs
Exenatide	152	748	2.57	8×10^{-7}
Sitagliptin	2	15	1.69	.37
Controls	32	405	—	—
		PANCREAS CANCER		
Drug	Pancreas cancer events	Control events	Odds ratio vs control drugs	*P*-value vs control drugs
Exenatide	81	1433	2.95	9×10^{-5}
Sitagliptin	16	306	2.72	.008
Controls	13	678	—	—
		THYROID CANCER		
Drug	Thyroid cancer events	Control events	Odds ratio vs control drugs	*P*-value vs control drugs
Exenatide	30	1433	4.73	4×10^{-3}
Sitagliptin	2	306	1.48	.65
Controls	3	678	—	—
		ALL OTHER CANCERS		
Drug	All cancer events	Control events	Odds ratio vs control drugs	*P*-value vs control drugs
Exenatide	375	1433	1.08	.47
Sitagliptin	59	306	0.8	.2
Controls	164	678	—	—

using R version 2.9 (The R Foundation for Statistical Computing).

Results

Control Events

The validity of the analysis is predicated on a similar rate of reported control events for each drug in the analysis. For the 2 test drugs and 4 control drugs, this was found to be the case. However, one drug initially chosen for the analysis (pioglitazone) had an elevated control event reporting rate compared to the other drugs, which were otherwise similar in their control event rate. This was not driven by any one of the controls, but rather was an overall elevation in reported control events. This means that either pioglitazone truly has an increased frequency of these events, or some reporting bias exists with pioglitazone relative to the other drugs. In either case, its inclusion in the analysis would be suspect. As a practical issue, despite the higher control event rate (control reports/total reports), the actual number of control reports was relatively low, and dropping it from the analysis resulted in only a modest reduction in the power of the analysis. The similarity of the control event rates for the remaining drugs supported the validity of this 2-level control analysis approach.

Pancreatitis. Exenatide and sitagliptin had similar patterns of reported pancreatitis events relative to the controls events. Pancreatitis has been reported >6-fold more frequently as an adverse event for patients administered exenatide (OR = 10.68; 95% confidence interval [CI]: 7.75–15.1; $P < 10^{-16}$) or sitagliptin (OR = 6.74; 95% CI: 4.61–10.0; $P < 10^{-16}$) when compared with other therapies (Table 1, Figure 1). When the adverse reporting events of the GLP-1 class of drugs (exenatide and sitagliptin) were considered together, the reported event rate of pancreatitis was approximately 10-fold greater than that of other therapies (OR = 9.99; 95% CI: 7.26–14.1; $P < 10^{-16}$).

Because of recent attention to the potential link between use of GLP-1 mimetic drugs and pancreatitis after the FDA's first warning in 2007[11] that pancreatitis appeared to be an adverse effect of exenatide treatment, the analysis was repeated using only events reported to have occured in 2006 or earlier. Because sitagliptin had only recently been made available at that time, there were insufficient reports to consider sitagliptin alone, so the event rates for the combined GLP-1 mimetic therapies of sitagliptin and exenatide were considered together. The reported event rate for pancreatitis for the GLP-1 mimetic drugs was still >2.5-fold increased compared to other therapies (OR = 2.55; 95% CI: 1.70–3.94; $P < 1 \times 10^{-6}$).

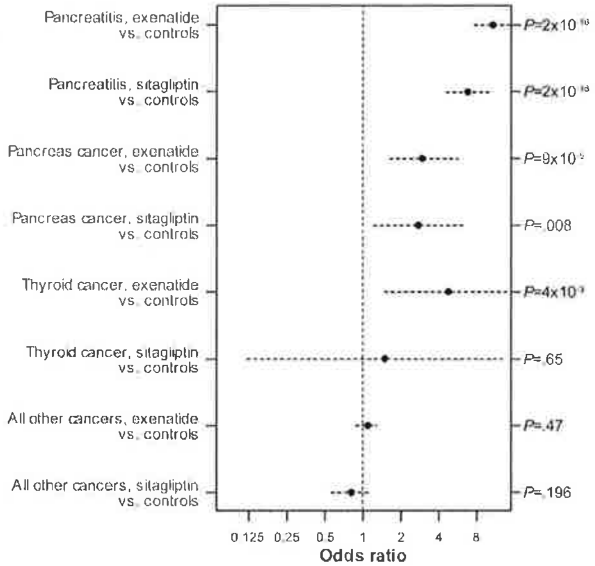

Figure 1. Odds ratio of test vs control events for exenatide, sitagliptin, and other therapies. The odds ratio of an adverse report of pancreatitis, pancreatic and thyroid cancer, or any cancer associated with exenatide and/or sitagliptin therapy vs other therapies.

Collectively, these data imply that there is an increased risk of pancreatitis in patients treated with either exenatide or sitagliptin vs the other therapies.

Pancreatic cancer. Because pancreatitis is a known risk factor for pancreatic cancer,[17] we evaluated the reported rates of pancreatic cancer with exenatide and sitagliptin compared to control events relative to rosiglitazone.

The reported event rate for pancreatic cancer was 2.9-fold greater in patients treated with exenatide compared to other therapies ($P = 9 \times 10^{-5}$). The reported event rate for pancreatic cancer was 2.7-fold greater with sitagliptin than other therapies ($P = .008$).

Thyroid cancer. Because thyroid tumors were reported to be increased in rodents treated with liraglutide in a filing to the FDA,[20] we examined the frequency of reported adverse events of thyroid cancer with the GLP-1 mimetic therapies vs rosiglitazone. The reported event rate for thyroid cancer in patients treated with GLP-1 mimetic therapy was increased and reached statistical significance in the exenatide group (OR = 4.73; $P = 4 \times 10^{-3}$), but not in the sitagliptin group (OR = 1.48; $P = .65$).

All other cancers. There has been a suggestion that DPP-4 inhibition may lead to impaired immune function and increased risk for cancers.[18,19] Therefore, we also examined the reported event rate for all other cancers (excluding pancreas and thyroid) associated with sitagliptin, exenatide, or the control therapies. Neither sitagliptin or exenatide were associated with a higher reported rate of other cancers. The risk for cancer increases with age but age was not different between the individuals in whom cancer (mean age, 61 years other therapies, 61 years exenatide, 64 years sitagliptin) or a control event (mean age, 62 years other therapies, 60 years exenatide, 63 years sitagliptin) was reported for the drugs included in this analysis.

Discussion

We report a >6-fold increased reported adverse event rate for pancreatitis with either of the first two GLP-1–based drugs available on the market in the United States, exenatide and sitagliptin, in this analysis of the FDA AERS database.

Analysis of the FDA AERS database is not the ideal mechanism to compare adverse event rates between drugs. Limitations of the FDA AERS database, including incomplete data and reporting biases, are well-known.[29] However, AERS has proven effective in similar earlier evaluations at detecting unintended drug side effects.[30-32] This analysis was undertaken notwithstanding these limitations, given the paucity of safety data available for this class of drugs, which is gaining a rapid increase in usage for a common disease. Randomized, controlled clinical trials remain the gold standard for such assessment. Those trials are typically powered for efficacy end points related to the relative attributes of the new drugs in accomplishing expected goals, such as glycemic control compared to previously available drugs. They do not necessarily accumulate sufficient data (in either patient numbers or follow-up) on infrequent or longer-term consequences of the drugs (eg, cancers). The primary goal of this study was to examine the FDA database as methodically as possible to establish whether there are sufficient grounds for concern that would indicate the need for studies that specifically examine the signals that arise in a prospective manner.

The approach we have taken should be robust against a range of potential reporting biases. In particular, if the test drugs have an overall increased reporting rate for events, the OR will be unaffected. Similarly, if the test events have an overall increased reporting rate, the OR will be unaffected. However, the approach has significant weaknesses. The analysis is retrospective. Potential confounders that influenced the choice of drug therapy for type 2 diabetes could introduce bias. For example if cigarette smokers were to be more likely treated with GLP-1 based therapy than other therapies for type 2 diabetes, a bias in favor of pancreatitis or pancreatic cancer would be introduced. Since cigarette smoking is not reported in the FDA data base we cannot exclude an unexpected bias in favor of diabetes treatment choice in this regard. More generally, the odds ratios reported here will be upwardly biased if patients who are at higher risk for pancreatitis, pancreatic and/or thyroid cancer received exenatide or stigiplatin, either as a first line therapy, or subsequent to a poor response to the therapy of first choice. There are plausible scenarios where this might happen, but we are unable to thoroughly determine the extent of this bias based on this retrospective study.

Also, although the controls (drugs and events) were prospectively defined, the analysis makes certain assump-

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tions about these controls that cannot be easily tested. One assumption is that the control events are not causally related to either the test drugs or the control drug. The events were chosen based on a review of available reported adverse event data for these drugs, but proving a negative is difficult. A second assumption is that, conditional on control event counts, the test events are not subject to reporting bias. That is, the control event counts serve as a surrogate for any differential reporting bias between the drugs. It is possible that alternate control drugs and/or alternate choices for control events could lead to different conclusions. However, we believed that restricting the analysis to prospectively defined controls and limiting the number of possible analyses would avoid many of the biases of a data-mining approach, given the large scope of the AERS database. To directly address this potential concern, we repeated the analysis using an alternate set of control events identified from the top events in the database. In all cases where the original analysis was significant that significance was maintained in the analysis using the alternate control events.

A potential confounding factor for the present analysis is obesity. The FDA AERS database does not record obesity (eg, body mass index), which is associated with pancreatitis risk[25] and may be associated with a higher usage of exenatide prescription due to the reported weight-loss effect of that drug. However, Blomgren et al report that, although statistically significant, the magnitude of the effect of higher body mass index on pancreatitis risk is equivalent to a 1.2-fold increased risk per 5 units of body mass index.[25] Given the fact that the FDA AERS database yields a >6-fold increased frequency of pancreatitis with either exenatide or sitagliptin treatment compared to other therapies, the potential confounding effects of obesity on the observed results is likely to be minimal.

Another potential confounder is gender. We performed gender stratified analysis for all of the comparisons between test drugs and control drugs; in all cases where the original analysis was significant, that significance was maintained in the gender stratified analysis, with no evidence of a confounding effect by gender on the reported odds ratio.

In contrast to the findings here, several studies recently reported no increase in pancreatitis in patients treated with GLP-1 receptor mimetic therapy.[22,33-35] These studies do not include any randomized controlled trials in which pancreatitis or pancreatic cancer were predefined end points, and that were adequately powered to address these questions. A retrospective study of pharmacy claims analysis found no increase in association between use of exenatide and pancreatitis compared to other antidiabetes drugs.[35]

Recent animal studies that also showed pancreatitis after GLP-1–based treatment provided some insight into the potential mechanisms by which this adverse event may be mediated.[14,15] GLP-1 receptors are abundantly expressed in the exocrine pancreas, and sitagliptin therapy has been shown to lead to increased pancreatic ductal replication, acinar to ductal metaplasia, and, less commonly, acute pancreatitis in a rat model of type 2 diabetes.[14] Increased ductal turnover and acinar to ductal metaplasia are both well-established characteristics of chronic pancreatitis in humans.[36] Low-grade chronic pancreatitis was noted in most rats treated with exenatide in one study,[15] but not in a subsequent study.[37] In the absence of human pancreas from individuals treated with GLP-1 mimetic drugs, it remains unknown if GLP-1–based therapy can induce asymptomatic low-grade pancreatitis. This is of concern because chronic pancreatitis increases risk of pancreatic cancer.[16,17,36]

For this reason, as a secondary analysis, we sought to address the question, does long-term GLP-1 therapy predispose to pancreatic cancer? At present there is no direct evidence to support an increase in pancreatic cancer with long-term GLP-1 therapy, but there are grounds for concern. Even though the drugs have only been available relatively recently, this analysis shows increased reported pancreatic cancer in association with either sitagliptin or exenatide treatment compared to other therapies. It might be argued that an apparent increase in pancreatic cancer with GLP-1 mimetic therapy is because pancreatic cancer is more frequent in type 2 diabetes,[28,38] but in the present analysis, this was controlled for by comparison with adverse reporting in association with control antidiabetic drugs, so all cases included presumably had type 2 diabetes. The selected control drugs have been reported as either neutral[13] or possibly even increasing the risk for pancreatic cancer.[27] We elected not to use metformin as a control because it has been reported to decrease the risk for pancreatic cancer.[26,39] We elected not to use insulin as a control because this would likely include controls with type 1 diabetes.

Because pancreatitis presumably acts as a risk factor for subsequent pancreatic cancer through the mechanisms of chronic inflammation and increased cell turnover,[36] it is not surprising that there is a progressive increased risk with years of exposure. For example, in patients with inherited chronic pancreatitis, the risk increases progressively with years of exposure, eventually reaching almost 75%.[17] The GLP-1–based drugs examined here have been on the market for no more than 6 years, raising the question of whether it is biologically plausible that there is already an increase in pancreatic cancer. Type 2 diabetes and obesity are known risk factors for chronic pancreatitis and pancreatic cancer, so it is reasonable to assume that in such individuals there is an increased incidence of the premalignant PanIN lesions in the pancreas. It has recently been proposed that these are derived from pancreatic duct glands that, in turn, might well be targets for GLP-1–induced proliferation.[40] It will be important to establish whether PanIN lesions and pancreatic duct glands express GLP-1 receptors and, if so, undergo proliferation in response to GLP-1 mimetic therapy. Such an effect could explain the relatively early signal for pancreatic cancer observed here.

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Because of thyroid tumors in mice treated with liraglutide reported to the FDA by Novo Nordisk,[20] we also examined the FDA AERS database for thyroid cancer in association with exenatide or sitagliptin therapy. There was an increase in reported thyroid cancer as an adverse event related to exenatide or sitagliptin therapy (data combined) compared to other therapies, this increase was statistically significant for exenatide. GLP-1 therapy has been shown to lead to C-cell hyperplasia in rats, but it is unknown what, if any, effects GLP-1 therapy has on the human thyroid gland.[20] The adverse reporting in the FDA database is not sufficiently sophisticated to robustly distinguish between thyroid cancer subtypes. It is perhaps of concern that this signal has appeared in the relatively short duration the drugs have been available when there was little a priori concern that would be expected to bias reporting. The findings for pancreatic and thyroid cancer reported here imply that more detailed studies of the actions of GLP-1 on the thyroid gland and exocrine pancreas in humans are warranted.

Finally, we examined the relative frequency of all other reported cancers as adverse events related to each of the 2 study drugs. This analysis was prompted by the reported actions of DPP-4 inhibition on the immune system and concerns raised that these might promote cancer through decreased immunosurveillance.[18,19]

Any action of DPP-4 inhibition to increase cancer risk might be expected to do so by permitting declaration of tumors previously held in check by an intact immune system.[18,19,41] As such, the effect may manifest early. To date these data do not identify a signal of other cancers as searched with either drug. Given the multiple search terms required for this analysis and the numerous variations that might be introduced in such a search, we fully acknowledge that this is the least secure analysis. While the prior analyses remained unchanged through the various changes in search requested in review, the all other cancers outcome did change according to changes in search.

In conclusion, analysis of the FDA adverse event reporting database suggests that the GLP-1 class of drugs being widely promoted for treatment of type 2 diabetes could have serious unintended and unpredicted side effects. Pancreatitis is >6-fold more likely to be reported in association with sitagliptin or exenatide than other therapy in type 2 diabetes. Despite the fact that exenatide and sitagliptin have been available for a relatively short period, it is of concern that, when taken together, there is a significantly increased association of thyroid cancer and pancreatic cancer with these therapies. The most obvious conclusion from these studies is that careful long-term monitoring of patients treated with GLP-1 mimetics or DPP-4 inhibitors is required. Almost all clinical trials of these drugs include metformin, the unchallenged first-line therapy of choice for type 2 diabetes. In contrast, in clinical practice in the field, the new drugs are being used as early monotherapies. Because metformin likely suppresses the putative actions of GLP-1 based drugs to promote pancreatitis and pancreatic cancer, it will be important to establish the impact of GLP-1 mimetic therapy in the absence of metformin in prospective clinical trials if this treatment is to be available for use in the absence of metformin. We agree with a recent proposal that such monitoring should be established independently of pharmaceutical companies.[29] For now this analysis of the FDA data base does not establish that pancreatitis, pancreatic and thyroid cancer are caused by GLP-1 based therapy. It simply raises the level of concern that they may be and that the appropriate prospective studies are required to rule them out.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2011.02.018.

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Reprint requests

Address requests for reprints to: Peter C. Butler MD, Larry L. Hillblom Islet Research Center, David Geffen School of Medicine at UCLA, 900 Veteran Avenue, 24-130 Warren Hall, Los Angeles, California 90095-7073. e-mail: pbutler@mednet.ucla.edu; fax: (310) 206-5368.

Conflicts of Interest

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CLINICAL PANCREAS

EXHIBIT 14

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ORIGINAL RESEARCH

Safety and Tolerability of Sitagliptin in Type 2 Diabetes: Pooled Analysis of 25 Clinical Studies

Samuel S. Engel · Elizabeth Round · Gregory T. Golm ·
Keith D. Kaufman · Barry J. Goldstein

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ABSTRACT

Introduction: In a previous pooled analysis of 19 double-blind clinical studies conducted by Merck, which included data available as of July 2009 on 10,246 patients with type 2 diabetes (T2DM), treatment with sitagliptin was shown to be generally well tolerated compared with treatment with control agents. As the sitagliptin clinical development program continues, additional studies with sitagliptin have been completed. The present analysis updates the safety and tolerability assessment of sitagliptin by examining pooled data from 25 double-blind clinical studies.

Methods: The present analysis included data from 14,611 patients in 25 studies with T2DM who received either sitagliptin 100 mg/day ($n = 7{,}726$; sitagliptin group) or a comparator agent ($n = 6{,}885$; non-exposed group). These studies represent all randomized, double-blind trials conducted by Merck that included patients treated with the usual clinical dose of sitagliptin (100 mg/day) for between 12 weeks and 2 years, and for which results were available as of December 2011. These studies assessed sitagliptin, versus comparator agents, taken as monotherapy, initial combination therapy with metformin or pioglitazone, or as add-on combination therapy with other antihyperglycemic agents (metformin, pioglitazone, a sulfonylurea ± metformin, insulin ± metformin, or metformin + pioglitazone or rosiglitazone). Patient-level data from each study were used to evaluate between-group differences in the exposure-adjusted incidence rates of adverse events (AEs).

Results: Overall incidence rates of AEs and drug-related AEs were higher in the non-exposed group compared with the sitagliptin group. Incidence rates of specific AEs were generally similar between the two groups, except for higher incidence rates of hypoglycemia related to the greater use of a

S. S. Engel (✉) · E. Round · G. T. Golm ·
K. D. Kaufman · B. J. Goldstein
Merck Sharp & Dohme Corp., Whitehouse Station, NJ, USA
e-mail: samuel.engel@merck.com

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sulfonylurea and diarrhea related to the greater use of metformin in the non-exposed group, and of constipation in the sitagliptin group. Treatment with sitagliptin was not associated with an increased risk of major adverse cardiovascular events, malignancy, or pancreatitis.

Conclusion: In this updated pooled safety analysis of data from 14,611 patients with T2DM, sitagliptin 100 mg/day was generally well tolerated in clinical trials of up to 2 years in duration.

Keywords: Adverse events; Dipeptidyl peptidase-4 inhibitor; Safety; Sitagliptin; Tolerability; Type 2 diabetes

INTRODUCTION

Since the introduction of sitagliptin into the diabetes therapeutic armamentarium in 2006, the use of dipeptidyl peptidase-4 (DPP-4) inhibitors for the management of hyperglycemia in patients with type 2 diabetes has increased worldwide. The role of DPP-4 inhibitors in diabetes treatment guidelines has similarly evolved, with the most recent American Diabetes Association (ADA)/ European Association for the Study of Diabetes (EASD) consensus guidelines considering DPP-4 inhibitors to be an appropriate second-line therapy after the initiation of metformin, and in the same category as other available antihyperglycemic therapies (including sulfonylureas, thiazolidinediones, glucagon-like peptide-1 (GLP-1) receptor agonists, and insulin) [1]. This represented a distinct departure from prior ADA/EASD guidelines, which considered only sulfonylureas and insulin to be "well-validated" second-line agents [2]. The emergence of the DPP-4 inhibitors has been driven in large part by the safety and tolerability profile of this class of agents compared with other antihyperglycemic agents. In particular, the low risk of hypoglycemia, the weight-neutrality, and the generally excellent tolerability when compared with other classes of drugs appear to have distinguished this class of incretin-based therapies.

In that context, it is important to continue to evaluate the safety and tolerability of this newer class of antihyperglycemic therapy in well-designed, randomized, controlled clinical trials. Recently, Monami et al. [3] performed an updated meta-analysis of 53 trials of at least 24 weeks in duration, which included over 33,000 patients with type 2 diabetes. In this analysis, which comprised 20,312 patients treated with a DPP-4 inhibitor and 13,569 patients treated with either placebo or an active comparator, outcomes of interest included the incidences of cancer, pancreatitis, all-cause and cardiovascular mortality, and major adverse cardiovascular events (MACE). There was no evidence of an increase in the incidence of cancer [Mantel–Haenszel odds ratio (MH-OR) 1.020, 95% CI 0.742, 1.402] or pancreatitis (MH-OR 0.786, 95% CI 0.357, 1.734) with DPP-4 inhibitor therapy. The overall MH-OR for all-cause and cardiovascular death in patients treated with DPP-4 inhibitor was 0.668 (95% CI 0.396, 1.124) and 0.505 (95% CI 0.252, 1.011), respectively. Additionally, a significantly lower risk of MACE (MH-OR 0.689, 95% CI 0.528, 0.899) was observed. While meta-analyses of published studies can provide an assessment of large numbers of patients across the class of DPP-4 inhibitors, the absence of patient-level data for specific adverse events and the focus, in most publications, on serious adverse experiences limit the ability of such analyses to provide a comprehensive assessment of the overall safety and tolerability profile of an individual DPP-4 inhibitor.

As part of the assessment of the safety and tolerability profile of sitagliptin, pooled analyses of patient-level clinical trial data have been previously reported [4–6]. This current pooled analysis includes data from 25 double-blind, randomized studies of sitagliptin 100 mg/day, and incorporates approximately 40% more patients and approximately 36% more patient-years of exposure than the prior pooled analysis. The availability of patient-level data coupled with a larger patient exposure allow for an enhanced ability to assess the incidence of less common adverse experiences, and also allow for more precise estimates of the incidence rates of reported adverse experiences.

METHODS

This post hoc analysis used a pooled population ($n = 14{,}611$) drawn from all 25 multicenter, US or multinational, double-blind, parallel-group studies conducted by Merck & Co., Inc., in which patients were randomized to receive sitagliptin 100 mg/day ($n = 7{,}726$) or a comparator ($n = 6{,}885$) for at least 12 weeks and up to 2 years (the duration of the longest studies) and for which results were available as of December 1, 2011 (complete study listing in Table 6 in Appendix). Each protocol was reviewed and approved by appropriate ethical review committees and authorities for each clinical site. All patients were to have provided written informed consent. The studies evaluated sitagliptin as monotherapy, initial combination therapy with either metformin or pioglitazone, or add-on combination therapy with other antihyperglycemic agents, including metformin, pioglitazone, a sulfonylurea (with and without metformin), insulin (with and without metformin), or metformin with rosiglitazone or pioglitazone. Patients not receiving sitagliptin (i.e., the non-exposed group) received placebo, metformin, pioglitazone, a sulfonylurea (with and without metformin), insulin (with and without metformin), or metformin with rosiglitazone or pioglitazone. From each contributing study, the pooling was conducted by including those portions of each study that had parallel treatment groups with concurrent exposures to sitagliptin 100 mg/day (primarily administered as 100 mg once daily) or other treatments (either placebo or active comparator). Studies conducted only in Japan were excluded from all analyses; a lower starting dose of sitagliptin has been separately developed in Japan. The pooling excluded studies conducted in patients with moderate-to-severe renal insufficiency, because these patients received sitagliptin at doses less than 100 mg/day. Studies describing the safety and tolerability of sitagliptin in patients with moderate and severe renal insufficiency have been previously published [7–9].

In each study, investigators were to report adverse events (serious and non-serious) that occurred during the conduct of the study, as well as serious adverse events occurring within 14 days following the last dose of blinded study drug. These events were encoded in a uniform manner using the Medical Dictionary for Regulatory Activities® (MedDRA version 14.1; MedDRA MSSO, Chantilly, VA, USA), in which terms for specific adverse events that are alike or pertain to the same organ system are categorized by System Organ Class (SOC). To account for potential differences between groups in duration of exposure to treatment, reports of adverse events are expressed as exposure-adjusted incidence rates (numbers of patients with events per 100 patient-years). These analyses were based on the time to the first (incident) event, calculated as follows: incident event rate = 100 × (total number of patients with ≥1 event during eligible exposure period per total patient-years of exposure). The incident event rate per 100 patient-years is referred to as the "incidence rate" throughout the manuscript. For those patients for whom an event was reported, the patient-years of exposure were calculated as the time from the first dose of sitagliptin (or comparator) at randomization to the time that the first post-randomization event occurred. For patients without an event, the patient-years of exposure were calculated as the time from the first dose to 14 days after the last dose of study medication (i.e., sitagliptin or comparator). Differences between treatment groups and the associated 95% CI were calculated using the Miettinen and Nurminen method, stratified by study [10]. For endpoints occurring in fewer than four patients in both groups, 95% CIs were not computed because they did not have the potential of excluding zero. No statistical adjustments were performed for multiple comparisons. All analyses were performed using SAS® version 9.1; SAS Institute, Inc., Cary, NC, USA.

The present analysis used patient-level data from each study to assess the incidence rates of adverse events that occurred following initiation of double-blind study drug. Many studies in this analysis included open-label glycemic rescue therapy, which was to have been initiated based on protocol-specified hyperglycemia criteria that were progressively stricter over the course of the study. When initiated, glycemic rescue therapy was added to the ongoing, blinded study medication to which patients had been randomized. Except where mentioned otherwise, the analyses presented below include all post-randomization events reported to have occurred during a given study, including those events with onset after the initiation of glycemic rescue therapy.

The analysis in this article is based on previously conducted studies, and does not involve any new studies of human or animal subjects performed by any of the authors.

Adverse Events of Interest

Hypoglycemia

For most studies, hypoglycemia was prespecified as an adverse event of interest. For all of the trials that were pooled for this analysis, hypoglycemia was based on investigator interpretation of clinical symptoms, without the requirement for a concurrent glucose determination. In contrast to the general analysis of adverse events, analyses of hypoglycemia adverse events excluded data following initiation of glycemic rescue therapy to avoid the confounding influence of medications that could cause hypoglycemia. In addition, a separate pooled analysis was performed including only those studies and portions of studies that did not include a sulfonylurea or insulin, to characterize the rate of hypoglycemia with sitagliptin relative to comparators not generally associated with an increased risk for hypoglycemia (i.e., metformin and pioglitazone, as well as placebo).

Gastrointestinal

The incidence of a composite endpoint of gastrointestinal (GI) adverse events (including diarrhea, nausea, vomiting, constipation, and a composite abdominal pain term, which included abdominal pain, upper and lower abdominal pain, abdominal and epigastric discomfort, and GI pain) was calculated. An additional analysis of these GI endpoints was

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conducted, excluding studies and portions of studies in which patients initiated metformin, to characterize the rate of these GI events with sitagliptin relative to comparators generally not associated with an increased risk for GI events. This separate analysis excluded data following initiation of glycemic rescue therapy.

MACE

An analysis of adverse cardiovascular events comprised of cardiovascular death in addition to ischemic events considered to be MACE was performed. For the MACE-related analysis, an exact method for Poisson processes [11], stratified by study, was used to calculate the exposure-adjusted incidence rate ratios (sitagliptin relative to comparator) and the associated 95% CI.

Neoplasms

All adverse event terms for neoplasms were reviewed in a blinded fashion and classified as corresponding to malignant or non-malignant neoplasms. All terms for malignant neoplasms were contained within the "Neoplasms benign, malignant, and unspecified" SOC, whereas terms for non-malignant neoplasms were contained both within and outside of the "Neoplasms benign, malignant, and unspecified" SOC. Incidence rates and between-group differences were computed for individual neoplasms as well as for the composite endpoints of all malignant neoplasms, all non-malignant neoplasms in the "Neoplasms benign, malignant, and unspecified" SOC, and all non-malignant neoplasms regardless of SOC.

Angioedema

Angioedema events and angioedema-related events, based on an expanded version of the Standard MedDRA Query (SMQ) that included anaphylactic reactions and hypersensitivity, were summarized by treatment group for the periods with and without exposure to an angiotensin-converting enzyme (ACE) inhibitor. Exposure to an ACE inhibitor was defined as the total days of use of an ACE inhibitor during the double-blind treatment period, with patients contributing to patient-years of exposure to an ACE inhibitor for the actual period of time that they were reported to have been taking an ACE inhibitor and to patient-years of non-exposure for the actual period of time that they were reported not to have been taking an ACE inhibitor.

Composite Endpoints of Interest

Incidence rates and between-group differences were calculated for a variety of composite endpoints, consisting of a collection of MedDRA adverse event terms related to the safety issue of interest. These composite endpoints included pancreatitis, pancreatic cancer, acute renal failure, proteinuria, bronchitis, pneumonia, upper respiratory infection, urinary tract infection, atrial fibrillation/flutter, and rash.

Laboratory Abnormalities

Percentages of patients meeting predefined laboratory abnormality criteria for liver enzyme abnormalities [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] and for serum creatinine were compared between groups.

RESULTS

Patient Characteristics and Exposure

In the entire 25-study cohort, patients (55% male) had an mean age of 54 years (range 19–91 years; 17% ≥65 years), a mean duration

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of diabetes of 5.1 years, and a mean glycosylated hemoglobin (HbA1c) of 8.4% at baseline (with 29% of patients having a baseline HbA1c ≥9.0%) (Table 1). The majority of patients were White (61%), with 18% Asian and 6% Black. At baseline, 10% of patients had a history of cardiovascular disease, and 81% had additional cardiovascular risk factors besides type 2 diabetes mellitus and cardiovascular disease, including hypertension (53%), history of dyslipidemia/hypercholesterolemia (49%), and history of smoking (39%). There were no meaningful differences between groups in these baseline characteristics.

The mean exposure to study drug was slightly greater in the sitagliptin group relative to the non-exposed group: 284 dosing days (range 1–791) and 264 dosing days (range

Table 1 Baseline characteristics

Characteristic	Sitagliptin (n = 7,726)	Non-exposed (n = 6,885)	Total (n = 14,611)
Gender, n (%)			
Male	4,196 (54)	3,788 (55)	7,984 (54.6)
Age, years	54.0 ± 10.3	54.4 ± 10.5	54.2 ± 10.4
Race, n (%)			
White	4,674 (60)	4,227 (61)	8,901 (61)
Black	427 (6)	384 (6)	811 (6)
Asian	1,436 (19)	1,227 (18)	2,663 (18)
Multiracial	462 (6)	427 (6)	889 (6)
Other or unknown	727 (9)	620 (9)	1,347 (9)
Body weight, kg	85.0 ± 19.6	85.8 ± 20.1	85.3 ± 19.8
Body mass index, kg/m^2	30.5 ± 5.7	30.7 ± 5.8	30.6 ± 5.7
HbA_{1c}, %	8.4 ± 1.3	8.4 ± 1.3	8.4 ± 1.3
Duration of T2DM[a], years	5.1 ± 5.4	5.1 ± 5.3	5.1 ± 5.4
On antihyperglycemic therapy, n (%)	3,001 (38.8)	2,773 (40.3)	5,774 (39.5)
History of CVD, n (%)	793 (10)	691 (10)	1,484 (10)
Patients with known CV risk factors other than T2DM and history of CVD, n (%)[b]	5,828 (81)	5,269 (82)	11,097 (81)
History of dyslipidemia, n (%)	3,862 (50)	3,356 (49)	7,218 (49)
History of hypertension, n (%)	4,110 (53)	3,666 (53)	7,776 (53)
History of smoking, n (%)[b]	2,712 (38)	2,539 (39)	5,251 (39)

Data are expressed as mean (± standard deviation) or frequency [n (%)], unless otherwise indicated

CV cardiovascular, *CVD* cardiovascular disease, *HbA1c* glycosylated hemoglobin, *T2DM* type 2 diabetes mellitus

[a] Excludes 16 patients (11 sitagliptin, 5 non-exposed) with unknown duration of diabetes

[b] Denominator is 7,177 for sitagliptin group and 6,451 for non-exposed group because history of smoking was not collected in all patients from Protocols 010, 014 and 074, and 11 patients from other studies did not provide information on smoking history

1–801), respectively. In the sitagliptin group, 2,457 (32%) patients were treated for at least 1 year, with 584 (8%) of these patients treated for 2 years; the corresponding numbers of patients in the non-exposed group were 1,775 (26%) and 470 (7%). The proportions of patients discontinuing treatment were 27.2% in the sitagliptin group and 28.8% in the non-exposed group.

Summary Measures of Adverse Events

The incidence rate of patients reporting one or more adverse events was higher in the non-exposed group compared with the sitagliptin group (Table 2). The incidence rate of drug-related adverse events was also higher in the non-exposed group, as was the incidence of patient discontinuations due to a drug-related adverse event; this was primarily due to the greater incidence rate of adverse events of drug-related hypoglycemia reported for the non-exposed group (data not shown). The incidence of serious adverse events was similar for the two groups, both overall (Table 2) and by SOC category (data not shown). The incidence of adverse events resulting in death, overall, was similar in the two treatment groups; for the Neoplasms SOC, however, the incidence of adverse events resulting in death was lower in the sitagliptin group compared with the non-exposed group (one event in 6,388 patient-years of follow-up compared with six events in 5,378 patient-years of follow-up, respectively, with a difference in rates of −0.1 events per 100-patient-years (95% CI −0.2, −0.0).

Incidence rates for adverse events in each SOC are in Table 3. There were three SOCs (Metabolism and nutrition disorders; Neoplasms benign, malignant, and unspecified; and Skin and subcutaneous tissue disorders) for which the 95% CI for the between-group

Table 2 Adverse event summary

	Incidence rate per 100 patient-years[a]		
	Sitagliptin	**Non-exposed**	**Difference between sitagliptin and non-exposed (95% CI)[b]**
≥1 adverse events	142.8	151.1	−7.6 (−13.9, −1.3)
With one or more drug-related[c] adverse events	19.1	25.5	−5.9 (−7.8, −4.1)
With one or more serious adverse events	7.3	6.9	0.4 (−0.6, 1.4)
With one or more serious drug-related[c] adverse events	0.4	0.2	0.1 (−0.1, 0.4)
Deaths	0.3	0.4	−0.1 (−0.4, 0.1)
Discontinuations due to adverse events	4.5	4.9	−0.5 (−1.3, 0.3)
Discontinuations due to drug-related[c] adverse event	1.6	2.2	−0.5 (−1.0, −0.0)
Discontinuations due to serious adverse event	1.7	1.4	0.2 (−0.2, 0.7)
Discontinuations due to serious drug-related[c] adverse event	0.2	0.1	0.1 (−0.0, 0.3)

[a] 100 × (number of patients with ≥1 event/patient-years of follow-up time)
[b] Between-group difference and 95% CI based on stratified analysis. Positive differences indicate that the incidence rate for the sitagliptin group is higher than the incidence rate for the non-exposed group. "−0.0" represents rounding of values that were slightly less than zero
[c] As determined by the investigator

Table 3 Summary of adverse event system organ classes

System organ class	Incidence rate per 100 patient-years[a]		
	Sitagliptin 100 mg	Non-exposed	Difference between sitagliptin and non-exposed (95% CI)[b]
Blood and lymphatic system disorders	1.2	0.9	0.2 (−0.1, 0.6)
Cardiac disorders	3.7	3.8	−0.2 (−0.9, 0.5)
Congenital, familial, and genetic disorders	0.2	0.2	−0.0 (−0.2, 0.1)
Ear and labyrinth disorders	1.6	1.9	−0.4 (−0.9, 0.1)
Endocrine disorders	0.3	0.4	−0.2 (−0.4, 0.0)
Eye disorders	3.8	3.9	−0.1 (−0.9, 0.6)
Gastrointestinal disorders	24.3	24.6	0.3 (−1.7, 2.3)
General disorders and administration site conditions	8.3	9.2	−0.9 (−2.1, 0.2)
Hepatobiliary disorders	1.2	0.9	0.2 (−0.1, 0.6)
Immune system disorders	0.9	0.9	−0.1 (−0.4, 0.3)
Infections and infestations	45.5	45.7	0.3 (−2.5, 3.1)
Injury, poisoning and procedural complications	8.8	8.8	0.3 (−0.9, 1.4)
Investigations	14.0	14.9	−1.3 (−2.7, 0.2)
Metabolism and nutrition disorders	11.1	17.5	−6.4 (−7.9, −4.9)
Musculoskeletal and connective tissue disorders	19.3	18.5	0.7 (−1.0, 2.4)
Neoplasms benign, malignant and unspecified	2.0	1.5	0.6 (−0.0, 1.2)
Nervous system disorders	15.1	14.7	0.3 (−1.1, 1.8)
Pregnancy, puerperium, and perinatal conditions	0.0	0.1	−0.0 (−0.1, 0.1)
Psychiatric disorders	4.3	4.5	−0.1 (−0.9, 0.6)
Renal and urinary disorders	2.8	2.6	0.1 (−0.5, 0.7)
Reproductive system and breast disorders	2.6	2.8	−0.2 (−0.8, 0.4)
Respiratory, thoracic and mediastinal disorders	7.9	8.0	−0.1 (−1.2, 0.9)
Skin and subcutaneous tissue disorders	7.8	6.7	1.1 (0.1, 2.1)
Social circumstances	0.0	0.0	−0.0[c]
Surgical and medical procedures	0.0	0.0	0.0[c]
Vascular disorders	5.4	5.3	−0.1 (−1.0, 0.7)

SOC system organ class

[a] 100 × (number of patients with ≥1 event in the SOC/patient-years of follow-up time)

[b] Between-group difference and 95% CI based on stratified analysis. Positive differences indicate that the incidence rate for the sitagliptin group is higher than the incidence rate for the non-exposed group. '0.0' and '−0.0' represent rounding for values that are slightly greater and slightly less than zero, respectively

[c] 95% CI were not computed for events that occurred in fewer than four patients in both groups, because the CI would necessarily have included 0

difference in incidence rates excluded 0. The between-group difference in the incidence rates of adverse events in the Metabolism and nutrition disorders SOC was primarily due to a higher incidence rate of hypoglycemia in the non-exposed group. The between-group difference in the Neoplasms benign, malignant, and unspecified SOC was related to a higher incidence rate in the sitagliptin group for non-malignant adverse events within the Neoplasms benign, malignant, and unspecified SOC, and was not the result of an imbalance in any single adverse event or any group of biologically related adverse events. The incidence rates of malignancy were similar for the two groups: 0.90 per 100 patient-years in the sitagliptin group and 0.93 per 100 patient-years in the non-exposed group [between-group difference of

−0.05 (95% CI −0.41, 0.30)]. For the Skin and subcutaneous disorders SOC, the three most common adverse events were rash, pruritus, and urticaria; the 95% CI included zero for all three of these adverse events.

Adverse Events of Interest

Hypoglycemia

The incidence rates of hypoglycemia were based on symptomatic reports of hypoglycemia, regardless of a concurrent glucose measurement. The predefined analysis for hypoglycemia (i.e., excluding data after initiation of glycemic rescue therapy) showed a between-group difference of −6.2 events per 100 patient-years (95% CI −7.6, −5.0), favoring the sitagliptin group. The difference observed for hypoglycemia was mainly due to the use of a sulfonylurea as a comparator agent in three studies of up to 2 years in duration, as well as a study in which patients were switched from placebo to a sulfonylurea during a double-blind continuation period (P020 in Table 6 in Appendix). Results from some individual studies included in this pooled analysis (in which sitagliptin was added to either a sulfonylurea with or without metformin or to insulin with or without metformin) demonstrated an increased risk for hypoglycemia with sitagliptin used in combination with these agents relative to placebo. In a separate pooled analysis of hypoglycemia in which confounding effects of a sulfonylurea or insulin as either background or comparator therapies were removed, the incidence rates of hypoglycemia were 5.6 and 5.1 per 100 patient-years in the sitagliptin ($n = 5{,}956$) and non-exposed ($n = 5{,}122$) groups, respectively, with a between-group difference of 0.5 events per 100 patient-years (95% CI −0.7, 1.6).

GI Symptoms

The primary analysis of select GI adverse events demonstrated similar incidence rates for the pooled select GI terms, the composite of abdominal pain terms, nausea, and vomiting (Table 4). The incidence rate of the adverse event of constipation was higher in the sitagliptin group (2.3) than in the non-exposed group (1.8). For the specific adverse event of diarrhea, a lower incidence was observed in the sitagliptin group. The differences observed for diarrhea mainly reflected the use of metformin as a comparator; when the confounding effects of initiation of metformin were removed, the incidence rates were 4.3 and 4.9 per 100 patient-years in the sitagliptin ($n = 5{,}940$) and non-exposed ($n = 5{,}122$) groups, respectively.

MACE

Detailed description of the analyses of MACE has been previously published [12]. The exposure-adjusted incidence of MACE was 0.65 per 100 patient-years in the sitagliptin group, and 0.74 per 100 patient-years in the non-exposed group, with an adjusted incidence rate ratio of 0.83 (95% CI 0.53, 1.30).

Neoplasms

As noted above, the analysis of all events of malignancies revealed similar incidences in the two treatment groups: 0.90 per 100 patient years in the sitagliptin group and 0.93 per 100 patient-years in the non-exposed group [between-group difference of −0.05 (95% CI −0.41, 0.30)]. Low incidence rates of a wide range of specific malignancies were reported, with similar rates in both treatment groups; the 95% CI did not exclude zero for any of the specific malignancies that were reported. The most common malignancies observed were basal cell carcinoma, prostate cancer, and

Table 4 Summary of composite adverse events/adverse events of interest

System organ class	Incidence rate per 100 patient-years[a]		
	Sitagliptin 100 mg	Non-exposed	Difference between sitagliptin and non-exposed (95% CI)[b]
Acute renal failure (narrow SMQ)	0.2	0.1	0.0 (−0.1, 0.2)
Acute renal failure (broad SMQ)	2.1	1.6	0.4 (−0.1, 0.9)
Atrial fibrillation/flutter	0.4	0.2	0.2 (−0.0, 0.4)
Bronchitis	4.0	3.5	0.5 (−0.2, 1.2)
Gastrointestinal adverse event composite	14.6	15.6	−0.5 (−2.0, 1.0)
Abdominal pain composite	3.7	4.0	−0.3 (−1.1, 0.4)
Constipation	2.3	1.8	0.6 (0.0, 1.1)
Diarrhea	6.6	8.4	−1.4 (−2.5, −0.4)
Nausea	2.8	3.2	−0.2 (−0.9, 0.4)
Vomiting	1.8	1.6	0.3 (−0.2, 0.8)
Pancreatitis	0.1	0.1	−0.0 (−0.2, 0.1)
Pancreatitis (including chronic pancreatitis)	0.1	0.1	0.0 (−0.1, 0.2)
Proteinuria	0.5	0.4	0.1 (−0.2, 0.3)
Pneumonia	0.9	0.8	0.2 (−0.2, 0.5)
Rash	1.7	1.1	0.6 (0.2, 1.1)
Upper respiratory infection	8.2	8.9	−0.6 (−1.7, 0.5)
Urinary tract infection	4.4	4.8	−0.3 (−1.1, 0.4)

SMQ standardized MedDRA queries

[a] 100 × (number of patients with ≥1 event/person years of follow-up time)

[b] Between-group difference and 95% CI based on stratified analysis. Positive differences indicate that the incidence rate for the sitagliptin group is higher than the incidence rate for the non-exposed group. "0.0" and "−0.0" represent rounding for values that are slightly greater and slightly less than zero, respectively

breast cancer (Table 8 in Appendix). Analyses were performed for the pool of terms representing the category of pancreatic cancer (adenocarcinoma of pancreas, pancreatic carcinoma, pancreatic carcinoma metastatic). The exposure-adjusted incidence rates for the pooled terms related to the category of pancreatic cancer were similar in the two treatment groups (0.05 and 0.06 events per 100 patient-years in the sitagliptin and non-exposed groups, respectively). The number of adverse events (three in each group) was below the pre-defined threshold for calculating a 95% CI.

The incidence rate of adverse events in the Neoplasms benign, malignant, and unspecified SOC overall was 2.03 per 100 patient-years in the sitagliptin group and 1.52 per 100 patient-years in the non-exposed group [between-group difference of 0.52 (95% CI 0.03, 1.01)]. The higher rate in the sitagliptin group was related to a higher rate of non-malignant neoplasms in the Neoplasms benign, malignant, and unspecified SOC [incidence rates of 1.18 and 0.60 per 100 patient-years in the sitagliptin and non-exposed groups, respectively; between-group difference of 0.60 (95% CI 0.25, 0.96)]. This difference was not the result of an imbalance in any single adverse event or any group of biologically related adverse events. The most common non-malignant neoplasm adverse event terms observed were uterine leiomyoma/leiomyoma, lipoma, and skin papilloma. The only term for which the 95% CI around the between-group difference excluded zero was lipoma [between-group difference 0.15 (95% CI 0.02, 0.29)]. A sensitivity analysis, performed to assess the incidence of non-malignant neoplasms across any SOC, revealed a similar pattern, with incidences of 1.58 and 1.12 per 100 patient-years in the sitagliptin and non-exposed groups, respectively [between-group difference of 0.45 (95% CI 0.02, 0.89)]; in this sensitivity analysis, the adverse event term "colonic polyp" was the most common, with similar incidences in the two treatment groups (0.25 and 0.26 per 100-patient years, respectively).

Angioedema

At baseline, 29.4% and 28.1% of sitagliptin-treated and non-exposed patients, respectively, were treated with ACE inhibitors. In the subgroup defined by ACE inhibitor use, the exposure-adjusted incidence of events was 0.99 per 100-patient-years in the sitagliptin group and 1.35 per 100-patient-years in the non-exposed group; for those patients not treated with ACE inhibitors, the incidence rates were 1.14 and 1.16, respectively.

Other Composite Endpoints

The following composite endpoints, primarily of interest due to theoretical mechanistic concerns and/or post-marketing case reports, were analyzed.

For the composite endpoint of pancreatitis (which included the MedDRA terms "pancreatitis" and "pancreatitis acute"), the incidence rates were similar for both groups (Table 4), with a difference in rate of −0.0 (95% CI −0.2, 0.1). A similar pattern was observed with an expanded composite that included the MedDRA term "pancreatitis chronic".

The incidence of acute renal failure was assessed using both the narrow SMQ and the broad SMQ (Table 4); low and similar rates were observed in both treatment groups for both composite endpoints, as well as for the composite endpoint of proteinuria, which comprised the MedDRA terms "albumin urine present" or "protein urine present".

Separate analyses were done on the composite endpoints of bronchitis, pneumonia, and upper respiratory infection (Table 4). Similar incidences were seen in both treatment groups for all three of these infection composites. Similar incidence rates were also observed for the composite endpoint of urinary tract infections (with or without cystitis).

The incidence of the composite endpoint of rash was higher in the sitagliptin group compared with the non-exposed group (Table 4). The difference in the composite endpoint was primarily related to a higher incidence of the individual terms "rash" and "rash macular".

The incidence of the individual adverse event term "atrial fibrillation" was higher in the sitagliptin group (Table 4). For the predefined composite endpoint of atrial fibrillation/atrial flutter, the between-group difference was 0.2 event per 100 patient-years, and the 95% CI did not exclude zero (95% CI −0.0, 0.4).

Specific Adverse Events for which CI Excluded Zero

The incidences of adverse events for which the 95% CI excluded zero are depicted in Table 5. There were 17 specific adverse events in which the incidence was higher in the sitagliptin group, and 23 specific adverse events in which the incidence was higher in the non-exposed group. For those adverse events in which the between-group difference was ≥0.5 events per 100 patient-years, there were two (constipation and dyspepsia) and seven (diarrhea, fatigue, edema peripheral, blood glucose decreased, hypoglycemia, blood glucose increased, and weight increased) in which the incidences were higher in the sitagliptin and non-exposed groups, respectively. Apart from the adverse event of hypoglycemia, the between-group differences in adverse events for which the 95% CI excluded zero were all less than 1.5 events per 100 patient-years.

Predefined Laboratory Abnormality Criteria

Liver Enzymes

The proportions of patients in the sitagliptin and non-exposed groups with their last measurement (obtained either at the time of discontinuation or at the final scheduled study visit) of AST ≥3 times the upper limit of normal (ULN) were both 0.3% [between-group difference 0.0 (95% CI −0.2, 0.2)]; the proportion of patients whose last ALT measurement was ≥3 times the ULN were 0.8% and 0.6%, respectively [between-group difference 0.0 (95% CI −0.0, 0.5)]. One patient in each group had a last value of ALT or AST ≥3 times the ULN with a simultaneous elevation of the total serum bilirubin ≥2 times the ULN.

Serum Creatinine

Similar proportions of patients had a last measurement of serum creatinine with an increase of ≥0.3 mg/dL (1.8% and 1.7% in the sitagliptin and the non-exposed groups, respectively). The proportions of patients who met the predefined criterion of two or more consecutive serum creatinine measurements with an increase from baseline of ≥0.3 mg/dL, or an increase from baseline of ≥50% were also similar in the two groups (0.8% and 0.6%, respectively).

DISCUSSION

An increase in the number of classes of antihyperglycemic therapy options available for the treatment of patients with type 2 diabetes offers patients more choices of effective and well-tolerated therapies that are needed for management of this chronic disease. Assessment of the risk/benefit profile of each class, and specific agents within each class, determines their value for patient management, and this has been acknowledged by the continued evolution of treatment guidelines [1]. Selective DPP-4 inhibitors, which provide physiologic increases in the incretins GLP-1 and gastric inhibitory polypeptide (GIP), offer the potential to be a preferred option for the management of hyperglycemia, since they lack many of the adverse effects observed with other diabetes medications (e.g., hypoglycemia, weight gain) [13]. Nevertheless, continued assessment of the safety and tolerability profile of newer agents, including DPP-4 inhibitors, is necessary as more patients are exposed to such treatments, both through expanded analyses of controlled clinical trial data as well as ongoing pharmacovigilance activities. While

Table 5 Adverse events for which the 95% confidence intervals around the difference in incidence rates excludes zero

Adverse event	Incidence rate per 100 patient-years[a]		
	Sitagliptin 100 mg	Non-exposed	Difference between sitagliptin and non-exposed (95% CI)[b]
Sitagliptin > non-exposed			
Acne	0.2	0.0	0.1 (0.0, 0.3)
Atrial fibrillation[c]	0.4	0.2	0.2 (0.0, 0.4)
Chest discomfort	0.3	0.1	0.2 (0.0, 0.4)
Constipation	2.3	1.8	0.6 (0.0, 1.1)
Dermatitis contact	0.6	0.3	0.3 (0.0, 0.6)
Dyspepsia	2.0	1.4	0.6 (0.0, 1.1)
Gilbert's syndrome	0.1	0.0	0.1 (0.0, 0.2)
Hepatomegaly	0.1	0.0	0.1 (0.0, 0.3)
Ischemic cardiomyopathy	0.1	0.0	0.1 (0.0, 0.2)
Lipoma	0.2	0.0	0.1 (0.0, 0.3)
Micturition urgency	0.1	0.0	0.1 (0.0, 0.2)
Ovarian cyst	0.1	0.0	0.1 (0.0, 0.2)
Periodontitis	0.3	0.1	0.2 (0.0, 0.3)
Rash macular	0.2	0.0	0.1 (0.0, 0.3)
Rash vesicular	0.1	0.0	0.1 (0.0, 0.2)
Tibia fracture	0.1	0.0	0.1 (0.0, 0.2)
Vaginal hemorrhage	0.1	0.0	0.1 (0.0, 0.2)
Non-exposed > sitagliptin			
Albumin urine present	0.0	0.2	−0.1 (−0.3, −0.0)
Blood glucose decreased	0.7	1.3	−0.5 (−0.9, −0.1)
Blood glucose increased	2.0	3.1	−1.1 (−1.8, −0.6)
Blood triglycerides increased	0.5	0.7	−0.3 (−0.6, −0.0)
Bradycardia	0.0	0.2	−0.2 (−0.3, −0.1)
Diarrhea	6.6	8.4	−1.4 (−2.5, −0.4)
Fatigue	1.6	2.1	−0.5 (−1.1, −0.0)
Hypoglycemia	6.7	13.0	−6.3 (−7.6, −5.1)
Hypoesthesia	0.7	1.0	−0.4 (−0.7, −0.0)
Neck pain	0.6	0.9	−0.3 (−0.7, −0.0)
Neurodermatitis	0.0	0.1	−0.1 (−0.2, −0.0)
Peripheral edema	2.2	3.0	−0.8 (−1.4, −0.2)

Table 5 continued

Adverse event	Incidence rate per 100 patient-years[a]		
	Sitagliptin 100 mg	Non-exposed	Difference between sitagliptin and non-exposed (95% CI)[b]
Pharyngeal erythema	0.0	0.1	−0.1 (−0.2, −0.0)
Sepsis	0.0	0.1	−0.1 (−0.2, −0.0)
Sinus headache	0.1	0.3	−0.2 (−0.4, −0.1)
Suicidal ideation	0.0	0.1	−0.1 (−0.2, −0.0)
Thrombophlebitis	0.0	0.2	−0.1 (−0.3, −0.0)
Urine ketone body present	0.0	0.1	−0.1 (−0.3, −0.0)
Weight increased	0.8	1.4	−0.6 (−1.0, −0.2)
White blood cell count increased	0.1	0.3	−0.2 (−0.4, −0.0)
Upper airway cough syndrome	0.0	0.1	−0.1 (−0.3, −0.0)
Vitreous detachment	0.0	0.1	−0.1 (−0.2, −0.0)
Wheezing	0.0	0.1	−0.1 (−0.3, −0.0)

[a] 100 × (number of patients with ≥1 event/patient-years of follow-up time)
[b] Between-group difference and 95% CI based on stratified analysis. Positive differences indicate that the incidence rate for the sitagliptin group is higher than the incidence rate for the non-exposed group. "0.0" and "−0.0" represent rounding for values that are slightly greater and slightly less than zero, respectively
[c] When atrial fibrillation and atrial flutter were combined, the between-group difference was 0.2 (95% CI −0.0, 0.4). Incidence rates for atrial flutter were 0.0 and 0.1 for the sitagliptin and the non-exposed groups, respectively, with a between-group difference of −0.1 (95% CI −0.2, 0.0)

pharmacovigilance activities, which include assessment of post-marketing adverse event reports, are of value in identifying potential safety signals, it is well-recognized that these voluntary, spontaneous adverse event reports are derived from a population of uncertain size; thus, it is generally not possible to reliably establish the incidence of such events or to establish a causal relationship between a medication and a specific adverse event. Assessment of the incidence of adverse events from randomized, controlled, clinical trials remains the gold standard for rigorous evaluation of potential safety issues.

Prior pooled analyses of randomized, controlled, clinical trials with sitagliptin, the first-marketed DPP-4 inhibitor, indicated that this agent was generally well tolerated in studies up to 2 years in duration. These data were generally consistent with subsequent pooled analyses of other DPP-4 inhibitors using patient-level data [14–16] as well as with meta-analyses of the DPP-4 class using study-level data [3, 17, 18].

In this current report, the safety and tolerability of sitagliptin was assessed in an expanded pool of studies that comprised over 14,000 patients, representing the largest patient-level data set published to date for a DPP-4 inhibitor. This updated analysis, which expanded on the prior analysis by the addition of six clinical trials, 4,365 patients and 3,114 patient-years of exposure, revealed that treatment with sitagliptin was generally well tolerated, with exposure-adjusted incidence rates of adverse events generally similar to those observed with control therapy that did not include sitagliptin or other DPP-4 inhibitor.

The attainment of currently recommended glycemic goals is limited, in large part, by the increased incidence of hypoglycemia seen with intensive therapies, and particularly with glucose-independent regimens, which include sulfonylureas and insulin. Incretin-based therapies, which provide a glucose-dependent mechanism for enhanced insulin secretion and reduced glucagon secretion, should theoretically be devoid of this risk. Consistent with this mechanistic consideration, the analysis of symptomatic hypoglycemia in studies in which sitagliptin was used as monotherapy or combination therapy (where there was no use of sulfonylureas or insulin) revealed similar rates of symptomatic hypoglycemia for sitagliptin-treated patients compared with non-exposed patients (who received either placebo, metformin, or pioglitazone as comparator agents). The incidence of symptomatic hypoglycemia was lower in the pooled sitagliptin-treated population, mainly related to the use of sulfonylureas as a comparator in several studies. As reported in several clinical trials, the addition of sitagliptin to regimens containing sulfonylurea or insulin resulted in an expected increase in the incidence of symptomatic hypoglycemia related to improvements in glycemic control and a general lowering of ambient glucose concentrations [19, 20]. These findings are consistent with those seen with other classes of antihyperglycemic agents that do not cause hypoglycemia when used as monotherapy, but do so when added onto sulfonylureas or insulin [21, 22]. Thus, in the context of combinations of antihyperglycemic therapies, the risk of hypoglycemia should be carefully considered in choosing appropriate treatment combinations.

An increase in the incidence of GI symptoms is characteristic of treatment with GLP-1 receptor agonists and with metformin. In the current pooled analysis, similar exposure-adjusted incidences were seen in both treatment groups for nausea, vomiting, a composite endpoint of terms related to abdominal pain, and a composite of diverse GI adverse events. Consistent with earlier pooled analyses [5, 6], there was a lower incidence of diarrhea and a higher incidence of constipation observed in the sitagliptin treatment group. These findings were, in part, related to the known effects of metformin on increasing the incidence of diarrhea. However, in a sensitivity analysis in which the confounding effects of metformin as a comparator was removed, a modest increase in the incidence of constipation was still observed. The mechanism underlying this observation is not understood; while DPP-4 inhibitors have not been observed to slow gastric emptying, it remains possible that the physiologic elevations in GLP-1 may have an impact on intestinal motility.

Interest in the relationship between antihyperglycemic agents and pancreatitis was triggered originally by post-marketing reports of acute pancreatitis in patients with type 2 diabetes treated with exenatide [23, 24]. Post-marketing reports of acute pancreatitis in patients treated with all currently marketed GLP-1 mimetics and DPP-4 inhibitors have been observed, and are noted in the labeled information for these products, including sitagliptin [25]. Post-marketing reports represent voluntary, spontaneous adverse event reports regardless of etiology or probability that the medication caused the adverse event. Additionally, post-marketing events are reported from a population of uncertain size; thus, it is generally not possible to reliably establish the frequency of such events or to establish a causal relationship between a medication and a specific adverse event. As noted by the US Food and Drugs Administration (FDA), spontaneous reports such as those contained in the FDA's Adverse Event Reporting System (AERS) database cannot be used to calculate the incidence of an adverse event [26]. Thus, an analysis of the AERS database that revealed an increase in the reporting rates for pancreatitis with sitagliptin and with exenatide is difficult to interpret, in part due to these intrinsic methodological limitations [27]. In a recently published analysis using a case–control study design, Singh et al. [28] reported a higher rate of hospitalizations for acute pancreatitis in patients with type 2 diabetes associated with the use of incretin-based therapies (sitagliptin or exenatide). This analysis has a number of methodological limitations, including the absence of data on pre-disposing baseline characteristics to allow for robust adjustment for confounding factors, a lack of confirmation of the diagnostic codes used, and lack of adjustment for potential channeling bias [29], which could result in preferential prescribing of incretin-based therapies to patients who were at greater risk for pancreatitis prior to treatment due to age, obesity or other risk factors. Randomized, controlled clinical trial data provide a more robust assessment of the incidence of adverse events. In the current pooled analysis, the incidence of acute pancreatitis was similar in the sitagliptin-treated and the non-exposed group, with exposure-adjusted incidence rates of 0.1 and 0.1 events per 100 patient-years, respectively. Similar findings were observed in the analysis of the composite endpoint of acute and chronic pancreatitis. These data are consistent with those reported previously in a smaller pooled analysis [4], and are also consistent with the systematic pharmacoepidemiologic retrospective cohort assessments performed in two large insurance claims databases [30, 31]. Events of pancreatitis will also be assessed in the sitagliptin cardiovascular outcome study TECOS [32], in which over 14,000 patients are currently enrolled; all cases of pancreatitis will be investigated by an adjudication committee (blinded to treatment assignment) using standard criteria for confirmation of the diagnosis of pancreatitis.

The relationship between antihyperglycemic therapies and malignancy has recently been a focus of increased attention. This is of particular importance in view of the reported association between both obesity and diabetes with an increased risk of malignancy [33], and recent associations of pioglitazone with bladder cancer [34], and dapagliflozin with bladder and breast cancer [35]. In the current pooled analysis, the exposure-adjusted incidence of malignancy was similar for sitagliptin-treated patients and non-exposed patients. The most common malignancies observed (basal cell carcinoma, prostate cancer and breast cancer) were reflective of the demographics of the population, and the incidence rates for these malignancies were similar in patients treated with sitagliptin and those not treated with sitagliptin. Of additional note was the similar incidence of pancreatic cancer in the two treatment groups. The relatively short-term duration of exposure (≤2 years) precludes definitive conclusions regarding any potential association with malignancy, but the lack of any signal in this randomized, controlled, clinical trial database is reassuring. Additionally, the incidence of cancer will be assessed in the long-term cardiovascular outcome study TECOS [32], in which a median duration of follow-up of 4 years is anticipated.

As had been observed in a previous pooled analysis [6], there was a slightly higher incidence of non-malignant neoplasms in the sitagliptin treatment group compared with the non-exposed group (1.18 versus 0.60 events per 100 patient-years). The between-group difference in incidence rates did not exclude zero for any non-malignant neoplasm other than lipoma. The most commonly observed non-malignant neoplasms (i.e., colonic polyp, uterine leiomyoma, and lipoma) were reflective of the expected pattern in the general adult population [36–38], and included a collection of disparate and diverse types of lesions of varying histology and biology. The large number of unrelated adverse event terms assessed in these pooled analyses and the varying and diverse histologies that underlie the reported non-malignant neoplasms suggest that the small increase in the incidence rate of non-malignant neoplasms in the sitagliptin group relative to the non-exposed group may be a stochastic finding and not related to the use of sitagliptin.

The incidence rate ratio of MACE in this pooled analysis was 0.83 (95% CI 0.53, 1.30). It is of interest that both preclinical and clinical mechanistic studies have demonstrated benefits of incretin-based therapies on cardiovascular function and outcomes [39, 40]. These data from the pooled analysis are consistent with a potential beneficial effect of sitagliptin on cardiovascular outcomes, but definitive evaluation of the cardiovascular effects of sitagliptin awaits the completion of the TECOS trial.

Over 17% of patients with diabetes are reported to have chronic kidney disease, and diabetes is associated with progressive renal insufficiency [41]. Clinical trials of sitagliptin in patients with moderate and severe renal insufficiency have indicated that sitagliptin is generally well tolerated in this population [7–9]. In this current pooled analysis of patients with normal or mildly impaired renal function, the evaluation of the impact of sitagliptin on renal function included an assessment of predefined changes in serum creatinine, and the incidence of adverse events related to progressive renal dysfunction (proteinuria and acute renal failure). For all of these measures, no difference between the two treatment groups was observed for the proportion of patients reaching the predefined laboratory abnormality thresholds or in the incidence of adverse events of proteinuria or acute renal failure.

The following are limitations of the present pooled analysis: the results are from patients included in randomized, controlled clinical studies of up to years in duration and, thus, may not be fully reflective of the use in the general population, nor of more prolonged use; the analysis focused on sitagliptin 100 mg/day, the usual clinical dose; and there were multiple comparisons made without an adjustment for multiplicity, which increased the chances for spurious findings. The strengths of these analyses include the ability to account for all reported adverse events using patient-level data, and the large number of clinical trials and patients analyzed.

CONCLUSION

In this updated pooled safety analysis based on data available as of December 2011 from over 14,000 patients with type 2 diabetes, treatment with sitagliptin 100 mg/day was generally well tolerated as monotherapy, as initial combination therapy, and as add-on therapy in double-blind, randomized clinical studies of up to 2 years in duration. Continued assessment of adverse events reported from clinical trials and from the post-marketing environment is ongoing.

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Conflict of interest. Samuel S. Engel, Elizabeth Round, Gregory T. Golm, Keith D. Kaufman, Barry J. Goldstein are employees of Merck Sharp & Dohme Corp. a subsidiary of Merck & Co., Inc, Whitehouse Station, New Jersey, and may own stock or stock options in the company.

Compliance with ethics guidelines. The analysis in this article is based on previously conducted studies, and does not involve any new studies of human or animal subjects performed by any of the authors.

APPENDIX

See Tables 6, 7, and 8.

Table 6 Studies and treatment arms included in the analysis

Study	Study design	Sitagliptin 100 mg/day group[a] (n = 7,726)	n	Non-exposed group[a] (n = 6,885)	n	References[b]
P010: b.i.d. dose-range finding	106-week active-controlled period	Sitagliptin 50 mg b.i.d. switched to sitagliptin 100 mg q.d.	122	Glipizide	123	[42]
P014: q.d. dose-range finding	12-week placebo-controlled period and 94-week active-controlled period	Sitagliptin 100 mg q.d.	110	Placebo (12 weeks) switched to metformin (94 weeks)	111	[43]
		Sitagliptin 50 mg b.i.d. switched to sitagliptin 100 mg q.d.	111			
P019: placebo-controlled add-on to pioglitazone study	24-week placebo-controlled period	Sitagliptin 100 mg q.d.	175	Placebo	178	[44]
P020: placebo-controlled add-on to metformin study	24-week placebo-controlled period and 80-week active-controlled period	Sitagliptin 100 mg q.d.	464	Placebo (24 weeks) switched to glipizide	237	[45]
P021: placebo-controlled monotherapy study	24-week placebo-controlled period	Sitagliptin 100 mg q.d.	238	Placebo	253	[46]
P023: placebo-controlled monotherapy study	18-week placebo-controlled period and 36-week active-controlled period	Sitagliptin 100 mg q.d.	205	Placebo (18 weeks) switched to pioglitazone (36 weeks)	110	[47]
P024: active-controlled add-on to metformin study	104-week active-controlled period	Sitagliptin 100 mg q.d.	588	Glipizide	584	[48, 49]
P035: placebo-controlled add-on to glimepiride, alone or in combination with metformin study	24-week placebo-controlled period and 30-week active-controlled period	Sitagliptin 100 mg q.d.	222	Placebo (24 weeks) switched to pioglitazone (30 weeks)	219	[20]
Study	**Study design**	**Sitagliptin 100 mg/day Group[a] (n = 7,195)**	n	**Non-exposed Group[a] (n = 6,267)**	n	**References[b]**
P036: placebo- and active-controlled study of initial combination use of sitagliptin and metformin	24-week placebo-controlled period; 80-week active-controlled period	Sitagliptin 100 mg q.d.	179	Placebo (24 weeks) switched to metformin (80 weeks)	176	[50–52]
		Sitagliptin 50 mg b.i.d. + metformin 500 mg b.i.d.	190	Metformin 500 mg b.i.d.	182	
		Sitagliptin 50 mg b.i.d. + metformin 1,000 mg b.i.d.	182	Metformin 1000 mg b.i.d.	182	
P040: placebo-controlled monotherapy study	18-week placebo-controlled period	Sitagliptin 100 mg q.d.	352	Placebo	178	[53]
P047: placebo-controlled monotherapy study in elderly patients	24-week placebo-controlled period	Sitagliptin 100 mg q.d.	91	Placebo	92	[54]
P049: active-controlled monotherapy study	24-week active-controlled period	Sitagliptin 100 mg q.d.	528	Metformin	522	[55]
P051: placebo-controlled add-on to insulin, alone or in combination with metformin study	24-week placebo-controlled period	Sitagliptin 100 mg q.d.	322	Placebo	319	[19]
P052: placebo-controlled add-on to metformin and rosiglitazone study	54-week placebo-controlled period	Sitagliptin 100 mg q.d.	170	Placebo	92	[56]

Table 6 continued

Study	Study design	Sitagliptin 100 mg/day Group[a] (n = 7,195)	n	Non-exposed Group[a] (n = 6,267)	n	References[b]
P053: placebo-controlled add-on to metformin study	30-week placebo-controlled period	Sitagliptin 100 mg q.d.	96	Placebo	94	[57]
P061: placebo- and active-controlled mechanism of action factorial study	12-week placebo-controlled period	Sitagliptin 100 mg q.d.	52	Pioglitazone	54	[c]
		Sitagliptin 100 mg q.d. + pioglitazone	52	Placebo	53	
P064: active-controlled study of initial combination use of sitagliptin and pioglitazone	54-week active-controlled period	Sitagliptin 100 mg q.d. + pioglitazone	261	Pioglitazone	259	[58, 59]
P066: active-controlled study of combination use of sitagliptin/metformin FDC	32-week active-controlled period	Sitagliptin 50 mg + metformin 1000 mg b.i.d. (FDC)	261	Pioglitazone 45 mg q.d.	256	[60]
P068: active-controlled study of sitagliptin and combination use of sitagliptin/metformin FDC	40-week active-controlled period	Sitagliptin 100 mg q.d. switched to sitagliptin 50 mg + metformin 1000 mg b.i.d. (FDC)	244	Pioglitazone 15 mg q.d. titrated up to 45 mg q.d.	247	[61]
P074: placebo-controlled add-on to metformin study	24-week placebo-controlled period	Sitagliptin 100 mg q.d.	197	Placebo	198	[62]
P079: active-controlled study of initial combination use of sitagliptin/metformin FDC	44-week active-controlled period	Sitagliptin 50 mg + metformin 1000 mg b.i.d. (FDC)	625	Metformin 1000 mg b.i.d. (FDC)	621	[63, 64]
P102: active-controlled study of initial combination use of sitagliptin and pioglitazone	54-week active-controlled period	Sitagliptin 100 mg q.d.	231	Pioglitazone 15 mg q.d.	230	[65]
		Sitagliptin 50 mg b.i.d. + pioglitazone 15 mg q.d.	230	Pioglitazone 30 mg q.d.	233	
		Sitagliptin 50 mg b.i.d. + pioglitazone 30 mg q.d.	231	Pioglitazone 45 mg q.d.	230	
		Sitagliptin 50 mg b.i.d. + pioglitazone 45 mg q.d.	230			
P128: placebo-controlled add-on to metformin and pioglitazone study	26-week placebo-controlled period	Sitagliptin 100 mg q.d.	157	Placebo	156	[66]
P801: placebo- and active-controlled add-on to metformin study	18-week placebo-controlled period	Sitagliptin 100 mg q.d.	94	Rosiglitazone 8 mg q.d.	87	[67]
				Placebo	91	
P803: active-controlled add-on to metformin study	30-week active-controlled period	Sitagliptin 100 mg q.d.	516	Glimepiride	518	[68]

b.i.d. twice daily, *FDC* fixed-dose combination tablet, *q.d.* once daily

[a] This column reflects the blinded treatment(s) to which patients were randomized. For studies identified in column 1 as "add-on" studies, all patients also received the active therapy indicated in column 1 (open-label)

[b] References are for the initial phases of the studies that had extension or continuation phases, unless a reference is provided for the results beyond the initial phase

[c] Alba et al. [69]

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Table 7 Adverse events with at least 1 incident event per 100 patient-years in one or both groups

Adverse event	Incidence rate per 100 patient-years[a]		
	Sitagliptin 100 mg	Non-exposed	Difference between sitagliptin and non-exposed (95% CI)[b]
Gastrointestinal disorders SOC			
Abdominal pain[c]	3.7	4.0	−0.3 (−1.1, 0.4)
Constipation	2.3	1.8	0.6 (0.0, 1.1)
Diarrhea	6.6	8.4	−1.4 (−2.5, −0.4)
Dyspepsia	2.0	1.4	0.6 (0.0, 1.1)
Gastritis	1.4	1.4	0.0 (−0.4, 0.4)
Gastroesophageal reflux disease	1.0	0.7	0.3 (−0.0, 0.7)
Nausea	2.8	3.2	−0.2 (−0.9, 0.4)
Toothache	1.1	1.3	−0.3 (−0.7, 0.1)
Vomiting	1.8	1.6	0.3 (−0.2, 0.8)
General disorders and administration site conditions SOC			
Fatigue	1.6	2.1	−0.5 (−1.1, −0.0)
Peripheral edema	2.2	3.0	−0.8 (−1.4, −0.2)
Infections and infestations SOC			
Bronchitis	3.7	3.3	0.5 (−0.2, 1.1)
Gastroenteritis	2.1	1.6	0.5 (−0.0, 1.0)
Influenza	4.0	4.7	−0.7 (−1.5, 0.0)
Nasopharyngitis	7.3	7.1	0.4 (−0.6, 1.4)
Pharyngitis	1.7	1.6	0.0 (−0.5, 0.5)
Sinusitis	2.3	2.4	−0.0 (−0.6, 0.5)
Upper respiratory tract infection	7.8	8.4	−0.5 (−1.6, 0.6)
Urinary tract infection	3.9	4.2	−0.3 (−1.1, 0.4)
Investigations SOC			
ALT increased	1.5	1.3	0.2 (−0.2, 0.7)
Blood glucose decreased	0.7	1.3	−0.5 (−0.9, −0.1)
Blood glucose increased	2.0	3.1	−1.1 (−1.8, −0.6)
Weight increased	0.8	1.4	−0.6 (−1.0, −0.2)
Metabolism and nutrition disorders SOC			
Hyperglycemia	1.4	1.6	−0.3 (−0.8, 0.2)
Hypoglycemia	6.7	13.0	−6.3 (−7.6, −5.1)
Musculoskeletal and connective tissue disorders SOC			

Table 7 continued

Adverse event	Incidence rate per 100 patient-years[a]		
	Sitagliptin 100 mg	Non-exposed	Difference between sitagliptin and non-exposed (95% CI)[b]
Arthralgia	3.3	3.6	−0.3 (−1.0, 0.4)
Back pain	4.2	3.9	0.2 (−0.5, 1.0)
Muscle spasms	1.1	1.3	−0.2 (−0.6, 0.2)
Musculoskeletal pain	1.5	1.5	−0.1 (−0.5, 0.4)
Myalgia	1.1	1.2	−0.1 (−0.5, 0.3)
Osteoarthritis	1.4	1.1	0.2 (−0.2, 0.6)
Pain in extremity	2.6	2.1	0.5 (−0.1, 1.0)
Nervous system disorders SOC			
Dizziness	2.6	2.6	−0.0 (−0.6, 0.6)
Headache	5.8	5.4	0.5 (−0.3, 1.4)
Hypoesthesia	0.7	1.0	−0.4 (−0.7, −0.0)
Paraesthesia	1.1	1.1	−0.1 (−0.5, 0.3)
Psychiatric disorders SOC			
Depression	1.3	1.2	0.2 (−0.2, 0.6)
Insomnia	1.4	1.3	0.1 (−0.4, 0.5)
Respiratory, thoracic, and mediastinal disorders SOC			
Cough	2.5	2.4	0.0 (−0.6, 0.6)
Oropharyngeal pain	1.2	1.1	0.1 (−0.3, 0.5)
Skin and subcutaneous tissue disorders SOC			
Rash	1.2	0.9	0.3 (−0.1, 0.7)
Vascular disorders SOC			
Hypertension	3.4	3.4	−0.1 (−0.8, 0.6)

ALT alanine aminotransferase, *SOC* system organ class

[a] 100 × (number of patients with ≥1 event/patient-years of follow-up time)

[b] Between-group difference and 95% CI based on stratified analysis. Positive differences indicate that the incidence rate for the sitagliptin group is higher than the incidence rate for the non-exposed group. "0.0" and "−0.0" represent rounding for values that are slightly greater and slightly less than zero, respectively

[c] Abdominal pain includes abdominal pain, upper and lower abdominal pain, and abdominal and epigastric discomfort

Table 8 Analysis of malignant neoplasms

Malignant neoplasm	Incidence rate per 100 patient-years[a]		
	Sitagliptin 100 mg	Non-exposed	Difference between sitagliptin and non-exposed (95% CI)[b]
Adenocarcinoma pancreas	0.00	0.02	−0.02
Astrocytoma malignant	0.00	0.04	−0.04
B-cell lymphoma	0.02	0.00	0.01
Basal cell carcinoma	0.14	0.19	−0.05 (−0.22, 0.11)
Bladder cancer	0.03	0.02	0.01
Bladder transitional cell carcinoma	0.02	0.00	0.02
Breast cancer	0.09	0.07	0.02 (−0.11, 0.15)
Carcinoid tumour of the small bowel	0.00	0.02	−0.02
Colon cancer	0.09	0.04	0.06 (−0.06, 0.19)
Diffuse large B-cell lymphoma	0.02	0.00	0.02
Endometrial cancer metastatic	0.00	0.02	−0.02
Fallopian tube cancer	0.00	0.02	−0.01
Gastric cancer	0.02	0.00	0.02
Glioblastoma multiforme	0.00	0.02	−0.02
Hepatic neoplasm malignant	0.02	0.02	−0.01
Hepatic neoplasm malignant non-resectable	0.00	0.02	−0.02
Laryngeal cancer	0.02	0.00	0.02
Lip and/or oral cavity cancer	0.02	0.00	0.02
Lung adenocarcinoma metastatic	0.00	0.02	−0.02
Lung carcinoma cell type unspecified stage IV	0.02	0.00	0.02
Lung neoplasm malignant	0.00	0.04	−0.04
Lung squamous cell carcinoma stage unspecified	0.02	0.00	0.02
Lymphoma	0.00	0.02	−0.02
Malignant melanoma	0.05	0.07	−0.02 (−0.14, 0.09)
Metastases to bone	0.03	0.02	0.01
Metastatic renal cell carcinoma	0.02	0.00	0.02
Myelodysplastic syndrome	0.00	0.02	−0.02
Non-small cell lung cancer	0.02	0.00	0.02
Oesophageal adenocarcinoma	0.00	0.02	−0.02
Oesophageal cancer metastatic	0.00	0.02	−0.02
Ovarian epithelial cancer	0.02	0.00	0.01

Table 8 continued

Malignant neoplasm	Incidence rate per 100 patient-years[a]		
	Sitagliptin 100 mg	**Non-exposed**	**Difference between sitagliptin and non-exposed (95% CI)[b]**
Pancreatic carcinoma	0.03	0.04	−0.01
Pancreatic carcinoma metastatic	0.02	0.00	0.01
Prostate cancer	0.11	0.07	0.04 (−0.10, 0.17)
Prostate cancer metastatic	0.00	0.02	−0.02
Prostate cancer stage III	0.00	0.02	−0.02
Rectal cancer	0.02	0.02	0.00
Renal cancer	0.02	0.00	0.02
Renal cell carcinoma	0.03	0.04	−0.01
Small cell lung cancer stage unspecified	0.02	0.00	0.02
Squamous cell carcinoma	0.02	0.04	−0.03
Squamous cell carcinoma of skin	0.08	0.02	0.06 (−0.04, 0.18)
Thyroid cancer	0.02	0.00	0.01
Uterine cancer	0.00	0.02	−0.02

[a] 100 × (number of patients with ≥1 event/patient-years of follow-up time)
[b] Between-group difference and 95% CI based on stratified analysis. Positive differences indicate that the incidence rate for the sitagliptin group is higher than the incidence rate for the non-exposed group CI was computed only for those endpoints with at least four patients having events in one or more treatment groups

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EXHIBIT 15

THE INTERNATIONAL JOURNAL OF CLINICAL PRACTICE

REVIEW ARTICLE

Sitagliptin: review of preclinical and clinical data regarding incidence of pancreatitis

S. S. Engel, D. E. Williams-Herman, G. T. Golm, R. J. Clay, S. V. Machotka, K. D. Kaufman, B. J. Goldstein

Merck Research Laboratories, Rahway, NJ, USA

Correspondence to:
Samuel S. Engel, MD, Merck Research Laboratories, 126 East Lincoln Avenue, Mail Code: RY34-A260, Rahway, NJ 07065-0900, USA
Email: samuel_engel@merck.com

Disclosures
All authors are employees of Merck & Co., Inc., the manufacturer of sitagliptin and may have stock or stock options in the company.

SUMMARY

Recent case reports of acute pancreatitis in patients with type 2 diabetes (T2DM) treated with incretin-based therapies have triggered interest regarding the possibility of a mechanism-based association between pancreatitis and glucagon-like peptide-1 mimetics or dipeptidyl peptidase-4 (DPP-4) inhibitors. The objective of this review was to describe the controlled preclinical and clinical trial data regarding the incidence of pancreatitis with sitagliptin, the first DPP-4 inhibitor approved for use in patients with T2DM. Tissue samples from multiple animal species treated with sitagliptin for up to 2 years at plasma exposures substantially in excess of human exposure were evaluated to determine whether any potential gross or histomorphological changes suggestive of pancreatitis occurred. Sections were prepared by routine methods, stained with haematoxylin and eosin and examined microscopically. A pooled analysis of 19 controlled clinical trials, comprising 10,246 patients with T2DM treated for up to 2 years, was performed using patient-level data from each study for the evaluation of clinical and laboratory adverse events. Adverse events were encoded using the Medical Dictionary for Regulatory Activities (MedDRA) version 12.0 system. Incidences of adverse events were adjusted for patient exposure. Tissue samples from preclinical studies in multiple animal species did not reveal any evidence of treatment-related pancreatitis. The pooled analysis of controlled clinical trials revealed similar incidence rates of pancreatitis in patients treated with sitagliptin compared with those not treated with sitagliptin (0.08 events per 100 patient-years vs. 0.10 events per 100 patient-years, respectively). Preclinical and clinical trial data with sitagliptin to date do not indicate an increased risk of pancreatitis in patients with T2DM treated with sitagliptin.

Review Criteria

An overview of the literature was performed to describe the prevalence and aetiology of pancreatitis. The effect of sitagliptin on pancreatic histology was evaluated in different species including mice, rats, dogs and monkeys. The incidence of pancreatitis with sitagliptin was analysed by pooling data from 19 controlled clinical trials with sitagliptin.

Message for the Clinic

The incidence of pancreatitis is increased in patients with type 2 diabetes (T2DM), and cases of pancreatitis have been reported in patients using most categories of antihyperglycemic medications. Recent postmarketing reports of pancreatitis in patients using incretin-based antihyperglycemic medications [i.e. the glucagon-like peptide-1 receptor (GLP-1R) agonist, exenatide and the dipeptidyl peptidase-4 (DPP-4) inhibitor, sitagliptin] have focused attention on this issue. Review of available preclinical and controlled clinical trial data do not indicate an increased risk of pancreatitis in patients treated with the DPP-4 inhibitor sitagliptin.

Introduction

Over the last decade, stimulation of glucagon-like peptide-1 (GLP-1) receptor-mediated signalling has been well validated as an approach for the treatment of type 2 diabetes (T2DM). The GLP-1 receptor agonists, GLP-1(7-36)-amide and GLP-1(7-37), hereafter collectively referred to as GLP-1, are produced and secreted from enteroendocrine L-cells of the intestinal epithelium. Key mechanisms responsible for glucose lowering by GLP-1 receptor agonism are stimulation of glucose-dependent insulin biosynthesis and secretion, inhibition of glucagon release and delayed gastric emptying.

Glucagon-like peptide-1 is rapidly hydrolysed *in vivo* ($t_{1/2} \sim$ 1–2 min) to produce a non-insulinotropic product, GLP-1(9-36) amide or GLP-1(9-37) (1). Dipeptidyl peptidase-4 (DPP-4), a serine dipeptidyl aminopeptidase that cleaves two N-terminal amino acids from GLP-1 to generate a non-insulinotropic peptide with no agonist activity against the GLP-1 receptor, is primarily responsible for this degradation. Because of the rapid proteolysis of GLP-1 by DPP-4, the native peptide is not suitable for therapeutic use. To overcome this problem, DPP-4-resistant GLP-1 receptor agonists were developed as injectable peptides for use in the treatment of T2DM. Exenatide (exendin-4), a GLP-1 mimetic discovered in lizard venom, was the first of these peptides approved for therapeutic use (2).

Pharmacological inhibition of DPP-4 is an alternate approach to increase the circulating concentrations of endogenous active GLP-1 (3). Multiple DPP-4 inhibitors have been identified and shown to

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stabilise endogenous active GLP-1 and improve glycaemic control in patients with T2DM. In addition to cleavage of GLP-1, DPP-4 has been shown to cleave multiple substrates *in vitro*, but few of these substrates have been validated as physiological substrates in humans. GLP-1 and another incretin, glucose-dependent insulinotropic polypeptide (GIP), are well-validated incretin substrates in humans, and both are rapidly metabolised to inactive peptides by the action of DPP-4. In mice, both GLP-1 and GIP have been shown to mediate the acute glucose lowering effects of DPP-4 inhibitor treatment in a glucose challenge paradigm (4). In patients with T2DM, however, because the insulinotropic effect of GIP may be diminished in this disease, DPP-4 inhibitors are believed to mediate glucose lowering primarily via stabilisation of GLP-1 (5).

Interest in the relationship between antihyperglycaemic agents (AHAs) and pancreatitis has recently emerged, triggered originally by reports of acute pancreatitis in patients with T2DM treated with exenatide (6,7). Initially described in a case report in 2006, subsequent postmarketing reports of acute pancreatitis in patients treated with exenatide as well as in patients treated with the DPP-4 inhibitor sitagliptin (8), the first DPP-4 inhibitor approved for use in patients with T2DM, have led to a focus on both the preclinical and clinical experiences with exenatide, other members of the GLP-1 agonist class, and the DPP-4 inhibitor class. In this review, we discuss the association of pancreatitis with T2DM, potential relationships between pancreatitis and medications other than sitagliptin used to treat patients with T2DM, and preclinical and clinical data on the incidence of pancreatitis in patients treated with sitagliptin.

Aetiology and epidemiology of pancreatitis in type 2 diabetes mellitus

The aetiologies of pancreatitis have been well described in numerous population studies (9). The most common inciting factors are gallstones (35–40%) and alcohol abuse (~30%) (10). Other risk factors for the development of acute pancreatitis include anatomic abnormalities, hypertriglyceridaemia, obesity, advancing age and use of drugs associated with pancreatitis. Patients with T2DM, who have a higher incidence of several of these known risk factors, have also been shown to have a higher incidence of pancreatitis relative to the general population. For example, in a multinational, placebo-controlled clinical trial involving nearly 10,000 patients with T2DM, the incidence of pancreatitis in the placebo group was 23 out of 4900 patients, or 0.47%, over 5 years (11), for an estimated incidence rate of 0.094 per 100 patient-years. In comparison, annual incidence rates of pancreatitis in the general population have been reported to range from 0.004 to 0.045 per 100 patient-years (12). A recently published study using retrospective claims data from the Ingenix® database, a large commercial US health plan, assessed the incidence of acute pancreatitis in a cohort of patients with T2DM; the reported incidence rate of 0.422 cases per 100 patient-years was greater than the rate of 0.149 cases per 100 patient-years observed in a cohort of general medical patients without diabetes [relative risk = 2.83 (95% CI: 2.61, 3.06)] (13). The rate of pancreatitis increased with age in the non-diabetes cohort, but remained relatively constant with advancing age in patients with T2DM. The reason(s) for the apparent higher risk of pancreatitis in patients with diabetes remains unclear, but may relate to the higher rates of known risk factors for pancreatitis, such as obesity, hypertriglyceridaemia, age and the greater use of medications potentially associated with pancreatitis in patients with T2DM.

Drug-induced pancreatitis

Drug-induced pancreatitis appears to be a relatively uncommon cause of pancreatitis, although the actual incidence is difficult to determine (14). The use of over 500 medications has been reported in patients with pancreatitis in the literature as anecdotal case reports, although the causal relationship of these medications to cases of pancreatitis remains unclear. This is due, in part, to incomplete information in the case reports regarding dose, time course of onset of pancreatitis in relation to initiation of the suspect medication, other confounding potential aetiologies and variable rechallenge experience. The interpretation of the aetiology of drug-induced pancreatitis in patients with T2DM may frequently be confounded by the concomitant use of medications that are commonly used in these patients and that have been associated with reports of pancreatitis, including statins and angiotensin-converting enzyme inhibitors (15).

Among published case reports of potential drug-induced pancreatitis, there are few regarding AHAs. Patients taking metformin have been reported to develop pancreatitis, although all but one case report involved an overdose or was in the setting of renal failure (16–19). In a case–control study conducted in Sweden between 1995 and 1998, the use of glyburide among patients with T2DM was associated with acute pancreatitis [adjusted odds ratio of 2.5 (95% CI 1.1–5.9)] (20). The use of other members of the sulphonylurea class in case reports of pancreatitis has included glimepiride and gliclazide (21,22). As described above, case reports of acute pancreatitis in patients treated with exenatide have also been published (6,23,24).

In contrast to the limited number of reports in the literature, a relatively higher number of postmarketing reports of pancreatitis associated with a broad range of AHAs has appeared in various databases. These postmarketing events are reported voluntarily from a population of uncertain size; thus, it is generally not possible to establish reliably the frequency of such events or to establish a causal relationship between a medication and a specific adverse event. The Adverse Event Reporting System (AERS), which replaced the Spontaneous Reporting System (SRS) in October 1997, is a computerised information database designed to support the U.S. Food and Drug Administration's (FDA) postmarketing safety surveillance program for all approved drug and therapeutic biological products. However, as noted on the FDA web site, 'AERS data do have limitations. First, there is no certainty that the reported event was actually due to the product. FDA does not require that a causal relationship between a product and event be proven, and reports do not always contain enough detail to properly evaluate an event. Further, FDA does not receive all adverse event reports that occur with a product. Many factors can influence whether or not an event will be reported, such as the time a product has been marketed and publicity about an event' (25). In particular, changes in reporting rates over time because of external factors (e.g. heightened interest in a specific adverse event related to reports of similar events with other medications or increased reporting rates for newly introduced medications) have been identified as significant factors that confound comparisons between medications regarding postmarketing reports of adverse events (26). Thus, it is generally understood that 'AERS cannot be used to calculate the incidence of an adverse event in the U.S. population' (25).

Despite these limitations, the AERS database provides a method to aggregate submitted postmarketing reports (27), which has resulted in recent updates to the prescribing information regarding postmarketing reports of pancreatitis for both exenatide and sitagliptin. In the context of the heightened interest regarding the association of GLP-1 receptor agonists and DPP-4 inhibitors with pancreatitis, a search of the AERS and SRS databases for reports of pancreatitis observed with other classes of AHAs was conducted by the authors, using data from 1968 through the third quarter of 2008. This analysis revealed reports of pancreatitis in patients using acarbose, chlorpropamide, exenatide, glimepiride, glipizide, insulin, metformin, miglitol, nateglinide, pioglitazone, pramlintide, repaglinide and rosiglitazone. A search of the same databases for commonly used AHAs (not including insulin), using data from 1968 through the first quarter of 2009, revealed cases of severe pancreatitis (i.e. haemorrhagic or necrotising) in which the following drugs were considered suspect therapy: acarbose, metformin, glimepiride, repaglinide, pioglitazone and rosiglitazone, in addition to exenatide and sitagliptin. Thus, pancreatitis in patients receiving AHAs in the postmarketing environment has been reported across a broad range of mechanistic categories and across the entire range of clinical severity. However, whether these reports are truly reflective of a relationship between the medications and the development of pancreatitis, or simply reflective of the increased rate of pancreatitis in the population of patients with T2DM, remains undetermined.

Pharmacoepidemiological studies can also be used to assess the incidence of postmarketing adverse events through the use of insurance or health system databases that comprehensively capture diagnostic and prescription information. In one such study, Dore et al. reported that the rates of acute pancreatitis among exenatide- or sitagliptin-treated patients were similar to those observed among metformin- or glyburide-treated patients (28). Similarly, Herrera et al. described similar rates of acute pancreatitis among patients prescribed exenatide, sitagliptin or other oral AHA therapies (29). While such data are reassuring, retrospective pharmacoepidemiological studies can be confounded by other factors (30). For example, interpretation of such analyses can be limited by the preferential channelling of patients to specific therapies, which can lead to a bias that cannot easily be adjusted for when interpreting results (31,32). Thus, controlled trials provide the most rigorous method for assessing the incidence of adverse effects of treatments.

Preclinical studies of sitagliptin

Extensive preclinical toxicity studies were performed as part of the sitagliptin development programme that informs on the occurrence of pancreatitis in a range of animal species: in rats, separate 2-week, 3-month, 6-month and 2-year studies comprising approximately 600 rats exposed to sitagliptin; in mice, separate 3-month and 2-year studies comprising approximately 550 mice exposed to sitagliptin; in dogs, 2-week, 3-month, 6-month and 1-year studies comprising 96 dogs exposed to sitagliptin as well as a 3-month study comprising 45 dogs exposed to the combination of sitagliptin and metformin; and in

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monkeys, a 3-month study comprising 24 cynomolgus monkeys exposed to sitagliptin (33).

In these studies in non-diabetic animals, sections from the pancreas were reviewed for potential pancreatic toxicity. In all species studied, the pancreas was carefully evaluated to determine whether any potential gross or histomorphological changes associated with administration of sitagliptin occurred. Sections were prepared by routine methods, stained with haematoxylin and eosin, and examined microscopically. Oral administration of sitagliptin for 3 months in monkeys, up to 12 months in dogs and up to 2 years in rats and mice was not associated with gross or histomorphological changes in the pancreas. There was no evidence of drug treatment-related acute pancreatitis in any species studied.

These preclinical toxicity studies were performed with doses that provided plasma exposures in excess of anticipated human exposures (based on the recommended dose of sitagliptin 100 mg/day), as measured by the 24-h area under the plasma concentration time curve (AUC_{0-24}). In the above studies, the highest dose tested in a 3-month study in rats was 2000 mg/kg/day, providing approximately a 271-fold margin over human exposure. In a 6-month study in rats, the highest dose studied was 180 mg/kg/day, providing approximately a 23-fold margin over human exposure. In the 2-year rat and mouse studies, the highest dose studied was 500 mg/kg/day, providing approximately a 56- and 68-fold margin, respectively, over human exposure. In dogs, the highest dose studied was 50 mg/kg/day, providing approximately a 28-fold margin over human exposure. In monkeys, the highest dose studied was 100 mg/kg/day, providing approximately a 28-fold margin over human exposure. Thus, at exposures well in excess of the expected human exposure, these preclinical studies did not reveal any evidence that administration of high doses of sitagliptin results in changes in the pancreas of non-diabetic rats, mice, dogs or monkeys.

A recent publication by Matveyenko et al. reported studies in which sitagliptin and metformin were administered orally to transgenic rats overexpressing human islet amyloid polypeptide (HIP) in the pancreas, a potential model of human T2DM (34). In one of these studies, 2-month old wild-type and HIP rats were fed a high-fat diet (HFD) and assigned to one of five groups (n = 7–9); wild-type (no drug), HIP rats (no drug), HIP rats administered sitagliptin (200 mg/kg/day), HIP rats administered metformin (200 mg/kg/day) and HIP rats administered sitagliptin (200 mg/kg/day) + metformin (200 mg/kg/day). Exposure levels in the HIP rats following a dose of 200 mg/kg/day of sitagliptin were not reported in this study but, based on previous data, this dose is likely to have produced exposures approximately 20-fold above exposures likely to occur in humans administered the recommended dose of sitagliptin 100 mg/day. Sitagliptin and metformin were administered orally for 12 weeks. In this study, upon histomorphological evaluation of the pancreas from these transgenic animals, it was noted that one of the 16 animals treated with sitagliptin, with or without metformin, had an area of pancreatitis. This area showed marked necrotising pancreatitis characterised by haemorrhagic necrosis, fibrosis, inflammatory cell infiltration and areas of ductal metaplasia. The authors stated that there were no observed effects in any HIP rats not treated with sitagliptin, and that pancreatitis was not observed in any of the other 89 HIP rats evaluated previously. However, the interpretation of this isolated finding is complicated by the limited amount of appropriate control data. The historical data referenced in the paper appears to include only approximately 13 HIP rats that were placed on HFD to induce insulin resistance and hyperglycaemia. Thus, in the historical 'control' database, the limited number of animals fed a HFD may have influenced the incidence of pancreatitis.

In contrast to the above findings of Matveyenko et al. (34), using the high-fat/streptozotocin murine model for T2DM in studies conducted at Merck Research Laboratories (35), no pancreatic histopathological effects were observed with sitagliptin treatment (33). To generate this model, 4-week-old male ICR mice were placed on a HFD in which 60% of energy intake is from fat. After 3 weeks of HFD, the mice are injected once with low-dose streptozotocin (90–100 mg/kg i.p.) to induce partial insulin deficiency. Three weeks after streptozotocin injection, the majority of HFD/streptozotocin-treated mice display hyperglycaemia, insulin resistance and glucose intolerance. The original purpose of this study was to explore the effects of sitagliptin on beta cell mass and function, and the primary results of the study have been recently published (36). In this study, fifty 10-week old mice were treated with sitagliptin at doses of up to 840 mg/kg/day for up to 10 weeks, resulting in estimated exposures (based on exposure data in CD-1 mice from a 14-week dose-range-finding study conducted to support the development of sitagliptin) as high as approximately 120-fold relative to the exposure in humans administered the recommended dose of 100 mg/day. Background changes of very slight focal chronic inflammation were seen in the pancreas in both control (N = 41) and streptozotocin-treated (N = 41) animals at similar incidences, with no difference noted in sitagliptin-treated animals (33). Of additional note is the study by Koehler et al., in which the effect of sitagliptin on the expression of

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genes associated with the development of pancreatitis in mice was compared with metformin and the GLP-1 receptor agonists exenatide and liraglutide (37). In contrast to the GLP-1 receptor agonists, neither sitagliptin nor metformin significantly altered pancreatic gene expression profiles. The same laboratory reported that in C57BL/6 mice ($N = 6$) treated with sitagliptin at doses as high as ~370 mg/kg/day, no histological evidence of pancreatitis was noted (D. Drucker, personal communication, University of Toronto, Toronto, ON, Canada). Another recent report described an increase in pancreatic acinar inflammation in Sprague–Dawley rats after chronic administration of exenatide 10 μg/kg (38), although the potential mechanism(s) responsible for this finding in this rat model remains speculative.

Thus, with the exception of a report of the histological findings in a single animal from a study of a genetically-altered rat model of diabetes, a broad range of preclinical studies in both non-diabetic and diabetic animals at exposures exceeding human exposure did not demonstrate a relationship between use of sitagliptin and the development of pancreatitis.

Clinical experience with sitagliptin

A previously published, pooled analysis of data from 12 double-blind, randomised clinical studies of up to 2 years in duration in patients with T2DM, comprising 6139 patients treated with either sitagliptin or a comparator agent (placebo or other AHA), was conducted to assess for differences in the incidence of adverse events between patients treated with sitagliptin and patients not exposed to sitagliptin (39). This pooled population included patients treated with the usual clinical dose of sitagliptin 100 mg/day (administered either as 100 mg q.d. or 50 mg b.i.d.) or concurrent control for between 12 and 106 weeks in clinical studies that were complete as of November 2007. Patients in the sitagliptin group ($N = 3415$) received sitagliptin when used as monotherapy, initial combination therapy with metformin, or add-on combination therapy with other AHAs including metformin, pioglitazone, a sulphonylurea (with and without metformin), or metformin + rosiglitazone. Patients in the control (non-exposed) group ($N = 2724$) received placebo, pioglitazone, metformin, a sulphonylurea (with and without metformin), or metformin + rosiglitazone. From each contributing study, the pooling was conducted by including portions of studies with controlled, parallel treatment groups. In this pooled analysis, no difference in the incidence of pancreatitis between patients treated with sitagliptin and patients not exposed to sitagliptin was observed (39).

To examine more comprehensively the safety and tolerability of sitagliptin, an updated pooled analysis of data from 19 double-blind, randomised clinical studies (including 7 additional studies relative to the prior pooled analysis) of up to 2 years in duration in patients with T2DM that were complete as of July 2009, and comprising 10,246 patients treated with either sitagliptin or a comparator agent (placebo or other AHA), was recently completed. Patients in the sitagliptin group ($N = 5429$) received sitagliptin (as either 100 mg q.d. or 50 mg b.i.d.) when used as monotherapy, initial combination therapy with either metformin or pioglitazone, or add-on combination therapy with other AHAs including metformin, pioglitazone, a sulphonylurea (with and without metformin), insulin (with and without metformin), or metformin + rosiglitazone. Patients in the non-exposed group ($N = 4817$) received placebo, pioglitazone, metformin, a sulphonylurea (with and without metformin), insulin (with and without metformin), or metformin + rosiglitazone. As in the prior pooled analysis, from each contributing study, the pooling was conducted by including portions of studies with controlled, parallel treatment groups.

This safety analysis used patient-level data from each study for the evaluation of clinical and laboratory adverse events. Adverse events were encoded using the MedDRA (Medical Dictionary for Regulatory Activities; version 12.0) system, a validated terminology database developed by the International Conference on Harmonisation. The specific MedDRA preferred terms used in this analysis were pancreatitis, acute pancreatitis and chronic pancreatitis. To account for the different exposures for the sitagliptin group compared with the non-exposed group, an exposure-adjusted analysis of incidence was conducted. For patients who had one or more events, person-time was computed beginning with the date of randomisation and ending with the date of the first event. For patients who did not have an event, person-time was computed beginning with the date of randomisation and ending 14 days after the last dose of study medication. Adverse events were expressed as exposure-adjusted incidence rates (i.e. number of patients with an event divided by patient-years of exposure). Differences in incidence rates between treatment groups were computed for all end-points, and the corresponding 95% confidence intervals (CIs) were calculated using the method of Miettinen and Nurminen (40), stratified by study. In most studies included in this analysis, glycaemic rescue therapy was to be implemented based upon protocol-specified hyperglycaemic criteria. Glycaemic rescue medications included metformin, pioglitazone, a sulphonylurea, or increased doses of insulin (in the

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Table 1 Person-time adjusted analysis of pancreatitis and pancreatitis acute adverse events including data after glycaemic rescue: sitagliptin 100 mg vs. non-exposed

Adverse event end-point	Treatment	*n*/Patient-years of exposure (100 patient-years event rate)*	Difference vs. non-exposed (95% CI)†
Pancreatitis/pancreatitis acute	Sitagliptin 100 mg	4/4708 (0.08)	−0.02 (−0.20, 0.14)
	Non-exposed	4/3942 (0.10)	
Pancreatitis	Sitagliptin 100 mg	3/4708 (0.06)	0.06 (−0.04, 0.19)
	Non-exposed	0/3943 (0.00)	
Pancreatitis acute	Sitagliptin 100 mg	1/4709 (0.02)	−0.08 (−0.25, 0.03)
	Non-exposed	4/3942 (0.10)	

n = Number of patients with ≥ 1 occurrence of the end-point.
*Patient-years of exposure were computed as the total time in the treatment period + 14 days for patients who did not have an event, and as the total time up to the time of the first event for patients who had an event.
†95% CI computed using the Miettinen & Nurminen method stratified by study.

add-on to insulin study). The analysis in this pooled safety population focused on the results that included data obtained both before and after a patient initiated rescue therapy.

As presented in Table 1, the incidence rate for the combined adverse events of pancreatitis and pancreatitis acute was similar for both groups (0.08 and 0.10 per 100 patient-years), with a between-group difference (95% CI) of −0.02 (−0.20, 0.14). For the specific events of 'pancreatitis acute' and 'pancreatitis', the 95% CI for the between-group difference in the event rates also included zero. For the adverse event of chronic pancreatitis, the event rates per 100 patient-years were 0.04 and 0.03 for the sitagliptin group and the non-sitagliptin-exposed group, respectively, with a between-group difference (95% CI) of 0.02 (−0.11, 0.13). In these clinical trials, there were no cases of haemorrhagic or necrotising pancreatitis, and no fatalities associated with pancreatitis were reported. Among the four patients in the sitagliptin group who had an adverse event of pancreatitis or pancreatitis acute, one had a prior medical history of recurrent pancreatitis, two had pancreatitis associated with gallstones and one had severe hypertriglyceridaemia. Among the four patients in the non-exposed group who had an adverse event of pancreatitis or pancreatitis acute, two had a prior medical history of chronic pancreatitis. Thus, this recent pooled analysis of 19 controlled clinical studies does not suggest an increased risk of pancreatitis in patients treated with sitagliptin.

Conclusion

Assessment of the safety of investigational and marketed drugs is an ongoing process that incorporates a variety of distinct, yet complementary, approaches. These approaches include, among others, preclinical studies in multiple species, typically involving drug exposures that greatly exceed the anticipated exposure in patients; controlled Phase I clinical studies in healthy subjects, also typically involving drug exposures that exceed the anticipated exposure in patients; controlled Phase II and Phase III clinical studies in the targeted patient population at therapeutic drug exposures; and postapproval analyses of clinical trial data, spontaneous postmarketing reports of adverse events and pharmacoepidemiological studies of large databases. As described in the present report, the preclinical and clinical trial data developed with sitagliptin to date do not indicate an increased risk of pancreatitis in patients with T2DM treated with sitagliptin. Nevertheless, as postmarketing events of pancreatitis have been reported for patients with diabetes while being treated with various AHAs, including sitagliptin, continued surveillance of the postmarketing experience and assessment of adverse events in patients participating in controlled clinical trials with sitagliptin are ongoing. Additional preclinical and clinical studies that are directed towards a better understanding of the potential relationship between specific medications, diabetes itself, and the incidence and severity of pancreatitis may lead to further knowledge in this area.

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EXHIBIT 16

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RESEARCH

Comparative safety and effectiveness of sitagliptin in patients with type 2 diabetes: retrospective population based cohort study

D T Eurich *associate professor*[1 2], S Simpson *associate professor*[2 3], A Senthilselvan *professor*[1], C V Asche *director*[4 5], J K Sandhu-Minhas *research associate*[2], F A McAlister *professor*[6 7]

[1]Department of Public Health Sciences, School of Public Health, University of Alberta, Edmonton, AB, Canada, T6G 2G3; [2]Alliance for Canadian Health Outcomes Research in Diabetes, University of Alberta, Edmonton, AB, Canada, T6G 2E1; [3]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada, T6G 2B7; [4]Center for Health Outcomes, University of Illinois College of Medicine at Peoria, Peoria, IL, 61656-1649, USA; [5]Department of Pharmacy Administration, University of Illinois at Chicago College of Pharmacy, Chicago, IL, 60612-7231, USA; [6]Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada, T6G 2B7; [7]Patient Health Outcomes Research and Clinical Effectiveness, University of Alberta, Edmonton, AB, Canada, T6G 2B7

Abstract

Objective To determine if the use of sitagliptin in newly treated patients with type 2 diabetes is associated with any changes in clinical outcomes.

Design Retrospective population based cohort study.

Setting Large national commercially insured US claims and integrated laboratory database.

Participants Inception cohort of new users of oral antidiabetic drugs between 2004 and 2009 followed until death, termination of medical insurance, or December 31 2010.

Main outcome measure Composite endpoint of all cause hospital admission and all cause mortality, assessed with time varying Cox proportional hazards regression after adjustment for demographics, clinical and laboratory data, pharmacy claims data, healthcare use, and time varying propensity scores.

Results The cohort included 72 738 new users of oral antidiabetic drugs (8032 (11%) used sitagliptin; 7293 (91%) were taking it in combination with other agents) followed for a total of 182 409 patient years. The mean age was 52 (SD 9) years, 54% (39 573) were men, 11% (8111) had ischemic heart disease, and 9% (6378) had diabetes related complications at the time their first antidiabetic drug was prescribed. 14 215 (20%) patients met the combined endpoint. Sitagliptin users showed similar rates of all cause hospital admission or mortality to patients not using sitagliptin (adjusted hazard ratio 0.98, 95% confidence interval 0.91 to 1.06), including patients with a history of ischemic heart disease (adjusted hazard ratio 1.10, 0.94 to 1.28) and those with estimated glomerular filtration rate below 60 mL/min (1.11, 0.88 to 1.41).

Conclusions Sitagliptin use was not associated with an excess risk of all cause hospital admission or death compared with other glucose lowering agents among newly treated patients with type 2 diabetes. Most patients prescribed sitagliptin in this cohort were concordant with clinical practice guidelines, in that it was used as add-on treatment.

Introduction

Glycemic control is considered one of the cornerstones in the management of type 2 diabetes. In addition to lifestyle changes, most patients will need glucose lowering treatment; most international guidelines recommend metformin as first line treatment.[1-3] Over the past few years, several new treatments have been introduced, most notably the new class of oral "incretin" drugs known as the dipeptidyl peptidase-4 (DPP-4) inhibitors. The DDP-4 inhibitors lower blood glucose by inactivating DPP-4, an enzyme responsible for metabolizing the gastrointestinal hormone glucagon-like peptide-1, which is responsible for augmenting the release of insulin in response to a rise in blood glucose.

Sitagliptin was the first DPP-4 inhibitor based treatment to be marketed in the United States in 2007, followed by saxagliptin in 2009. DPP-4 inhibitors are considered weight neutral and have been shown to modestly improve modulators of cardiovascular risk, including triglycerides, low density lipoprotein cholesterol, high density lipoprotein cholesterol, and blood pressure; however, the data are relatively inconsistent across studies.[4-6] Several pooled safety analyses have suggested potential benefits associated with DPP-4 inhibitors. A recent meta-analysis of 18 phase III randomized controlled trials reported that DDP-4 inhibitors were associated with a 52% (95% confidence interval 0.31% to 0.75%) relative risk reduction in

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Correspondence to: D T Eurich 2-040 Li Ka Shing Center for Health Research Innovation, University of Alberta, Edmonton, AB, Canada, T6G 2E1 deurich@ualberta.ca

major adverse cardiovascular events (cardiovascular death, non-fatal myocardial infarction or acute coronary syndrome, stroke, arrhythmias, and heart failure) compared with other active or placebo treatment.[7] However, no evaluation of broader outcomes of interest to clinicians such as all cause death or all cause hospital admissions were reported. To date, evidence on the potential benefits or risks of DPP-4 inhibitors is lacking, and, given recent experiences with other novel glucose lowering treatments such as thiazolidinediones, concerns exist.[8 9]

Although several studies assessing specific safety endpoints (pancreatitis, upper respiratory tract infections, renal failure) have been done,[10-13] to our knowledge no large comparative effectiveness studies have evaluated sitagliptin, the most widely prescribed and longest marketed DPP-4 inhibitor in the United States, in "real world" patients with broader outcomes including all cause hospital admissions or mortality. We thus designed this study to compare outcomes associated with sitagliptin treatment compared with other glucose lowering agents. We hypothesized that the use of sitagliptin would not be associated with increased risk of hospital admission, mortality, or cardiovascular events.

Methods

We did a population based retrospective cohort study using a large US claims and integrated laboratory database that included employed, commercially insured patients with dependants from all 50 states (Clinformatics Data Mart, OptumInsight Life Sciences Inc). Patient level data are collected directly from the clinical encounter, providing a unique, clinically rich source of information. The database has been widely used and includes de-identified longitudinal data on patients, including administrative and demographic data (type of insurance plan, sex, age, dates of eligibility, income) and all billable medical service claims including inpatient and outpatient visits and medical procedures (de-identified physician and facility identifier, date and place of service, cost of service, admission and discharge dates, procedure and diagnosis codes), all laboratory tests and results (lipids (high density lipoprotein cholesterol, low density lipoprotein cholesterol, triglycerides), renal function (creatinine, proteinuria), liver function, blood glucose (glycated hemoglobin), complete blood count, and so on), and pharmacy claims data (de-identified prescribing physician, drug dispensed based on national drug codes, quantity and date dispensed, drug strength, days' supply, cost of service).[14-17] All clinical diagnoses are recorded according to ICD-9-CM (international classification of diseases, 9th revision, clinical modification) codes and procedure codes (according to ICD-9 and current procedural terminology 4 codes). The database contains more than 13 million annual lives, and data are updated every 90 days. We de-identified and accessed the data by using protocols compliant with the Health Insurance Portability and Accountability Act.

Cohort selection

We identified an inception cohort of new users of oral antidiabetic drugs between the index years of January 1 2004 and December 31 2009. We defined new users as those with no prescription records for any antidiabetic drug, including insulin, for one year before their index date (that is, the date of the first claim for their antidiabetic drug).[18-20] To be included, all patients had to be at least 20 years of age on the index date, be enrolled in a commercial medical insurance plan, and have one year of continuous medical insurance before the index date (fig 1). We excluded patients starting insulin as their first antidiabetic agent, but progression from oral antidiabetic treatment to insulin was allowed during follow-up. We subsequently followed all patients until death, termination of medical insurance, or December 31 2010, providing a maximum follow-up of six years. As saxagliptin was released only in July 2009, and few patients were using it in our inception cohort (n=610), we excluded these patients from our primary analysis, although we included them in a sensitivity analysis.

Our primary outcome was all cause hospital admission or death. We also analyzed each component of the composite endpoint (all cause death or all cause hospital admission) separately. In addition, we evaluated the effect of sitagliptin on cardiovascular related hospital admissions (ICD-9-CM codes 410, 411.1, 428, 430-438) and the combined endpoint of cardiovascular related hospital admission or all cause mortality. For the composite outcomes, we used time to the first event (either admission date or date of death) as the dependent variable (failure time) in our main analysis. Patients who did not reach the outcome of interest were censored at their study exit date. We ascertained vital status through linkage to the US national death index file.[21] This is considered to be highly valid and reliable for matching of death, with greater than 98% sensitivity when social security number data are available, as in our case.[22] We did not have access to cause of death in the data.

Analysis

As the patterns of glucose lowering treatment are quite complex, we used time varying Cox proportional hazards regression to estimate more precisely the effect of exposure to drug. In these analyses, we set time zero at the start of the first oral antidiabetic drug use.[23] We established time varying exposure to oral antidiabetic drugs and insulin on the basis of the expected duration of each prescription by using the "days' supplied" field in the prescription drug dispensations database.[24] We considered patients to be exposed to the drug of interest unless they did not refill their prescription for two consecutive periods (based on the days' supply field) of the previous prescription. We then considered patients as unexposed to the drug of interest for the period of time from the end of the first consecutive period to the end of the study or until they restarted the drug. Subsequent definitions whereby we considered participants as non-exposed immediately after the expected duration and definitions that allowed for a 14 day "grace period"[24] did not appreciably change our results and are otherwise not presented. We attributed outcome events to the drugs the patient was expected to be receiving at the time of the event, and we assumed no legacy or carryover effects from remote exposure to any of the glucose lowering drugs for the primary analysis, although we assessed legacy effects in sensitivity analyses.

Exposure to antidiabetic drugs

For the primary exposure of interest, for each day of follow-up, we classified exposure to antidiabetic drugs into six categories that were not mutually exclusive: any sitagliptin use, any metformin use, any sulfonylurea use, any thiazolidinedione use, other oral antidiabetic drug use (acarbose, meglitinides, pramlintide), and any insulin use. For analyses, we included each drug exposure class in the model as a dummy variable with the reference group being no exposure to the drug of interest (for example, exposure to sitagliptin compared with no exposure to sitagliptin, after adjustment for use/non-use of other antidiabetic drugs). We classified patients receiving combination pills (such as sitagliptin and metformin) as receiving both agents concurrently (that is, any sitagliptin use and any metformin use). In addition, we specifically evaluated the potential effects of sitagliptin in subgroups at high risk, such as those with a history of ischemic heart disease or those with estimated glomerular filtration rate below 60 mL/min at the index date. Secondarily, as current clinical practice guidelines recommend that sitagliptin should be used as add-on treatment,[1 3] we specifically evaluated the effects of sitagliptin used in combination. Thus, we further classified drug exposure into mutually exclusive categories of combination treatment and included this variable in our model as our exposure of interest: sitagliptin plus metformin, sitagliptin plus a sulfonylurea, and metformin plus a sulfonylurea. For these analyses, the metformin plus sulfonylurea combination treatment group served as our reference category (usual guideline recommended care).[1 3]

Confounding variables

In addition to the time varying exposure to oral antidiabetic drugs and insulin, we included numerous additional confounding variables in the Cox regression models as time fixed variables in the analyses on the basis of the most recent value within one year before starting glucose lowering treatment, as sitagliptin has been shown to alter several potential risk factors.[5] The specific variables included were age, sex, socioeconomic status (type of medical insurance and median household income according to the 2010 US census[25]), clinical laboratory data (glycated hemoglobin, high density lipoprotein cholesterol, low density lipoprotein cholesterol, triglycerides, estimated glomerular filtration rate (according to the modified diet in renal disease calculation: ≥90, 89.9-60, 59.9-30, <30 mL/min), albuminuria, hemoglobin concentrations), and prescription drugs (for example, antiplatelet drugs, anticoagulants, statins, calcium channel blockers, β blockers, angiotensin converting enzyme inhibitors/angiotensin receptor blocker, renin inhibitors, diuretics, nitrates). For patients who did not have specific clinical laboratory data measured, we used the missing indicator approach for all analyses.[26] To further control for comorbidities, we used the adjusted clinical groups score derived from the Johns Hopkins ACG system, version 9,[27] which is a single comorbidity score weighted by the 32 adjusted diagnostic groups that performs equally to or better than the Charlson and Elixhauser comorbidity scores.[28] In addition, we included the expanded diagnosis cluster for diabetes to further control for diabetes specific complications.[27] We also included adherence to glucose lowering treatments in our models by using the medication possession ratio based on the prescription days' supply field.

To further control for confounding by indication, we used several techniques. Firstly, as we have previously done,[29] assuming that sicker patients are more likely to be admitted to hospital, we included the total number of hospital admissions in the year before the index date as a covariate in analyses. Secondly, from the Johns Hopkins ACG system, we included the total number of chronic conditions identified as a marker of global comorbidity, as well as a medically frail condition marker as derived by the system (for example, any occurrence of malnutrition, abnormal weight loss, morbid obesity, dementia, falls, decubitus ulcer).[27] Lastly, as others have, we included a time varying propensity score whereby we updated the propensity or probability of receiving sitagliptin every three months throughout the follow-up period by using all available data to date.[30] The propensity score contained almost 60 variables, including demographic variables (age, sex, age-sex interaction, state, type of insurance), socioeconomic factors (income), comorbidities, health service use, laboratory data, markers of frailty, and drug treatments. We observed no clinically important differences in covariates within fifths of the propensity score between patients exposed to sitagliptin and those not exposed. For example, for patients in the highest propensity fifth, background use of both metformin (82% v 83%) and sulfonylureas (32% v 31%) was very similar for sitagliptin users and non-users.

Sensitivity analyses

To evaluate the robustness of our results, we did several additional analyses. Firstly, as sitagliptin was not available until 2007, we restricted our new user cohort to the years of 2007-09. Secondly, we excluded all patients using insulin treatment, as insulin may be viewed as a marker for more advanced disease, and repeated our analyses. Thirdly, we modified our definition of exposure whereby we considered patients as unexposed to the drug of interest for all future periods if they stopped using the drug for at least two consecutive periods (that is, restarting of drugs was not allowed). Fourthly, we evaluated any "legacy" effects by considering patients as exposed in all future time periods if they had any previous exposure to the drug of interest. Fifthly, we censored patients if they stopped all antidiabetic drugs (including insulin) for at least two consecutive periods. Sixthly, we combined sitagliptin use with users of saxagliptin (n=610) to evaluate the overall effect of DDP-4 inhibitors. Seventhly, we evaluated the association between sitagliptin use and acute pancreatitis or upper respiratory tract infections, which have been reported in the literature.[10 11 13] Lastly, we repeated our main analyses using a high dimensional propensity score that uses an algorithm to empirically identify candidate covariates. This method creates and prioritizes potential confounders by using all available diagnostic codes, procedural codes, hospital admissions, drugs, and laboratory values. From this exhaustive list, the top 500 most influential covariates on the association of interest are selected to generate the propensity score. Use of the high dimensional propensity score has been shown to improve effect estimates compared with predefined covariate based propensity scores.[31]

Results

Between 2004 and 2009, 72 738 new users of oral antidiabetic drugs met the inclusion and exclusion criteria (fig 1⇓). The average age was 52 (SD 9) years, 54% were men, 10% had a history of ischemic heart disease, and 9% had diabetes related complications (table 1⇓). We identified 8032 (11%) patients who used sitagliptin at any point during the study. Although statistical differences existed between sitagliptin and non-sitagliptin users owing to the large numbers, few clinically important differences existed with the exception that sitagliptin users tended to have higher use of insulin treatment and higher rates of diabetes related complications (table 1⇓). Among sitagliptin users, most (7293; 91%) used sitagliptin as an add-on treatment with other oral agents, consistent with current clinical practice guidelines.

By the end of follow-up (182 409 patient years with a mean duration of 2.5 (SD 1.7) years), our primary composite endpoint had occurred in 14 215 (20%) patients: 14 121 (19%) patients were admitted to hospital at least once, and 520 (1%) died (table 2⇓). Users of sitagliptin had lower crude incident rates of all cause hospital admission or all cause mortality compared with other antidiabetic agents. However, in time varying multivariable Cox regression analysis, sitagliptin users had similar hazards for the primary composite endpoint to sitagliptin non-users after adjustment for the use of other glucose lowering strategies, demographics, and clinical and comorbidity data (adjusted hazard ratio 0.98, 95% confidence interval 0.91 to 1.06) (table

2⇓). Similarly, sitagliptin users had a similar risk to non-users for the combined endpoint of cardiovascular related hospital admissions or all cause mortality (adjusted hazard ratio 0.92, 0.79 to 1.07), all cause mortality (1.14, 0.79 to 1.65), all cause hospital admissions (0.98, 0.91 to 1.06), and cardiovascular related hospital admissions (0.90, 0.77 to 1.07) (table 2⇓; fig 2⇓).

Among patients with a history of ischemic heart disease, sitagliptin users had a similar risk to non-users for the primary composite endpoint (adjusted hazard ratio 1.10, 0.94 to 1.28), the combined endpoint of cardiovascular related hospital admissions or mortality (0.99, 0.77 to 1.27), all cause mortality (1.02, 0.53 to 1.99), all cause hospital admissions (1.10, 0.94 to 1.28), and cardiovascular related hospital admissions (0.98, 0.76 to 1.27). Similarly, in those with estimated glomerular filtration rate below 60 mL/min, sitagliptin users had a similar risk to non-users for the primary composite endpoint (adjusted hazard ratio 1.11, 0.88 to 1.41), the combined endpoint of cardiovascular related hospital admissions or mortality (0.86, 0.34 to 1.37), all cause mortality (0.99, 0.34 to 2.89), all cause hospital admissions (1.10, 0.87 to 1.40), and cardiovascular related hospital admissions (0.92, 0.57 to 1.50) (fig 2⇓).

Compared with users of metformin plus a sulfonylurea, users of sitagliptin plus a sulfonylurea had a similar risk for our primary composite endpoint (adjusted hazard ratio 1.03, 0.76 to 1.39), whereas use of sitagliptin plus metformin was associated with lower risk (0.82, 0.72 to 0.93). Subsequent post hoc analyses in which we restricted our entire cohort to only new users of metformin (n=55 678), which is recommended first line treatment for most patients with type 2 diabetes, confirmed these results: adjusted hazard ratio 0.85, 0.74 to 0.98 for addition of sitagliptin to metformin compared with addition of a sulfonylurea to metformin. However, an analysis of only new users of sulfonylureas as first line treatment did not show any difference between those patients who switched to sitagliptin plus metformin and users of sulfonylurea who added metformin (adjusted hazard ratio 1.04, 0.71 to 1.53).

Restriction of cohort entry to begin in 2007 did not materially change our results for sitagliptin use compared with non-use for the primary combined endpoint (adjusted hazard ratio 1.00, 0.91 to 1.10). Analyses excluding insulin users produced nearly identical results to our main findings on use of sitagliptin for the composite endpoint (adjusted hazard ratio 1.01, 0.94 to 1.09). Our results were also robust to changes in the definition of exposure whereby we considered patients as unexposed if they stopped the drug of interest for at least two consecutive periods (that is, no restarts allowed) (adjusted hazard ratio 0.97, 0.90 to 1.05), as they were to consideration of a legacy effect of any previous exposure (0.97, 0.91 to 1.04) and censoring of patients after they discontinued all drugs, including insulin, for at least two consecutive periods (0.99, 0.91 to 1.06). The inclusion of the 610 patients using saxagliptin provided nearly identical results to those observed with sitagliptin alone (adjusted hazard ratio 0.98, 0.91 to 1.05). We also found no association between the use of sitagliptin and the risk of acute pancreatitis (adjusted hazard ratio 1.10, 0.68 to 1.77) or the risk of acute upper respiratory tract infections (P=0.97) or pancreatic cancers (P=0.96) compared with sitagliptin non-users. Finally, the inclusion of a high dimensional propensity score did not change any of our estimates materially (adjusted hazard ratio 1.02, 0.95 to 1.10 for sitagliptin users compared with sitagliptin non-users for the primary combined endpoint).

Discussion

In our large clinically rich population, we found that the use of sitagliptin was not associated with any appreciable excess risk of all cause hospital admission or all cause mortality in a broad spectrum of patients with newly treated diabetes or in higher risk groups such as those with a history of ischemic heart disease or with reduced kidney function. Importantly, we also did not observe any safety "signals" related to cardiovascular related hospital admissions or death, supporting the premise that sitagliptin seems to be safe in patients with diabetes, at least if used in the manner in which it was used in this cohort. To our knowledge, this is the first study to evaluate the comparative effectiveness and safety of sitagliptin, or any of the DPP-4 inhibitors, at the population level. Given the current controversy about other antidiabetic agents, most notably the thiazolidinediones, this is important information for patients and for clinicians managing blood glucose concentrations in patients with diabetes.

Comparison with other studies

Although this is the first population based study assessing the effect of DPP-4 inhibitors on mortality and cardiovascular events, our results are broadly consistent with previous observational studies that have shown that sitagliptin is not associated with an increased risk of acute pancreatitis[10 11]; however, unlike previous studies,[13] we did not find any association with upper respiratory tract infections, although previous estimates of upper respiratory tract infections may have substantial reporting bias.[13] Our results are not consistent with recent meta-analyses of published and unpublished randomized controlled trials reporting that various DDP-4 inhibitors (alogliptin, dutogliptin, linagliptin, saxagliptin, sitagliptin, and vildagliptin) are associated with statistically significant 30-60% reductions in major adverse cardiac events and non-significant 33% and 48% reductions in all cause and cardiovascular death compared with other active drugs or placebo treatment.[7 32 33] Importantly, these analyses included studies of relatively short duration and that enrolled highly selected patients. Although modulation of the glucagon-like peptide-1 system has been shown to have pleiotropic effects on the cardiovascular system,[6 34] we did not observe any significant benefits, or risk, at the population level. However, our analyses also suggest that sitagliptin was prescribed in our cohort for patients with more advanced diabetes, given the higher rates of complications of diabetes at baseline and higher glycated hemoglobin values. Thus, despite the use of time varying propensity scores, any potential beneficial effects of sitagliptin on morbidity and mortality may have been masked by the higher baseline risk of patients prescribed sitagliptin in our cohort.

Combination treatment

Our results also suggest that differences may exist between the use of sitagliptin in combination with metformin and the use of sitagliptin in combination with sulfonylureas. Whether this effect is truly related to the use of sitagliptin as opposed to a metformin effect is uncertain. A large body of observational data on the use of metformin has consistently shown that users of metformin have lower morbidity and mortality rates than sulfonylurea users.[19 20 35 36] However, in our cohort, metformin users tended to have better glycemic control at baseline and less comorbidity and were less likely to use additional treatment, so our results may simply represent residual confounding. On the other hand, we found that those metformin treated patients prescribed sitagliptin as add-on treatment had better outcomes than those prescribed a sulfonylurea as add-on treatment. As metformin is recommended first line treatment for most patients with type 2 diabetes, our results may have important implications for the selection of add-on treatment in these patients. However, we acknowledge that these analyses were post hoc and exploratory in nature and should be considered hypothesis generating rather than definitive.

Strengths and limitations of study

Despite several strengths of our study, including the availability of detailed clinical data (such as glycated hemoglobin, cholesterol, and markers of renal function), the use of advanced statistical techniques including time varying propensity scores, and the relatively large sample size of new users of antidiabetic agents, several limitations are inherent to our work. Firstly, and most importantly, this is an observational study and any results must be interpreted with caution. Our results may be attributed to selection bias in that physicians may have given or withheld sitagliptin in patients perceived to be at varying degrees of risk, which even time varying propensity scores cannot fully adjust for. Secondly, we were not able to fully adjust for unmeasured confounders such as blood pressure or body weight. For example, sitagliptin may have been reserved for heavier patients because of its neutral effect on weight gain. However, this selection bias would actually strengthen our conclusions on the safety of sitagliptin, as it would have biased towards higher event rates in sitagliptin users; thus, if anything, our results would have underestimated any potential benefits of sitagliptin on morbidity or mortality. Moreover, although we did not have actual blood pressure measurements, we did include physician assigned diagnosis of hypertension or related comorbidities, as well as all major blood pressure lowering drug classes, in both our propensity score and adjusted analyses. A third limitation is that our population largely consisted of middle aged patients with commercial health insurance. Fourthly, as DPP-4 inhibitors have only recently been marketed, our study had a relatively short follow-up duration (mean 2.5 years). Although no short term adverse events were noted with sitagliptin, the longer term safety of sitagliptin cannot be fully elucidated yet. Finally, we did not have data on other potential adverse outcomes not requiring admission to hospital and thus cannot comment on the safety of sitagliptin with respect to these endpoints.

Conclusions

Our results suggest that clinicians have rapidly adopted the use of sitagliptin in the management of type 2 diabetes, but in most cases it is being used as add-on treatment rather than initial monotherapy (congruent with guidelines during the time of our study).[1-3] Initial evidence from phase III clinical trials and pre-clinical data suggest cardiac benefits with DPP-4 inhibitors, but we did not observe any clinically important effects in newly treated patients with type 2 diabetes. Although results of the ongoing Trial Evaluating Cardiovascular Outcomes With Sitagliptin (TECOS) are needed to definitively assess the safety of sitagliptin in patients with diabetes and cardiovascular disease, the trial is not scheduled to report for several years and will not cover the comparative effectiveness and safety of sitagliptin in the broader population with diabetes. Until then, our observational data provide evidence of the comparative effectiveness and safety of this agent and support the recommendations in current clinical practice guidelines to use sitagliptin as needed in people with diabetes.[1 3]

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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: The study was approved by the ethics review board of the University of Alberta, Edmonton, Alberta, Canada, and the New England Institutional Review Board, United States.

Data sharing: No additional data available.

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What is already known on this topic

Several pooled safety analyses have suggested potential benefits associated with dipeptidyl peptidase-4 inhibitors

No large published studies have evaluated the effect of sitagliptin on broad outcomes such as all cause hospital admissions or mortality in "real world" patients

What this study adds

Sitagliptin was not associated with any appreciable excess risk of all cause hospital admission or all cause mortality in a broad spectrum of patients with newly treated diabetes

Nor was it associated with excess risk in higher risk groups such as patients with a history of ischemic heart disease or with reduced kidney function

These observational data provide evidence of the comparative safety of sitagliptin and support current recommendations to use sitagliptin as add-on treatment if needed in people with diabetes

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Tables

Table 1| Baseline characteristics according to drug use at any time during follow-up. Values are numbers (percentages) unless stated otherwise

Characteristics	No sitagliptin exposure (n=64 706)	Exposed to sitagliptin (n=8032)	Exposed to metformin (n=61 979)	Exposed to sulfonylurea (n=22 470)	P value*
Mean (SD) age (years)	52.4 (9.5)	52.2 (9.1)	52.0 (9.4)	52.1 (9.5)	0.20
Male sex	35 008 (54)	4565 (57)	33 127 (53)	13 284 (59)	<0.001
Mean (SD) income ($)	48 152 (6060)	48 371 (6235)	48 199 (6083)	47 953 (5867)	<0.01
Type of insurance:					
Point of service	39 124 (60)	4887 (61)	37 727 (61)	13 073 (58)	<0.001
Exclusive provider	11 435 (18)	1471 (18)	11 053 (18)	4129 (18)	
Preferred provider	6446 (10)	958 (12)	6403 (10)	2620 (12)	
Health maintenance	7088 (11)	650 (8)	6243 (10)	2444 (11)	
Independent	610 (1)	65 (1)	550 (1)	204 (1)	
Other	3 (0)	1 (0)	3 (0)	0 (0)	
Clinical parameters at baseline					
Mean (SD) adjusted diagnostic groups comorbidity score	8 (9)	8 (9)	8 (9)	9 (9)	0.36
History of cardiovascular disease:					
Ischemic heart disease	7213 (11)	898 (11)	6345 (10)	2569 (11)	0.93
Heart failure	1638 (3)	210 (3)	1314 (2)	748 (3)	0.66
Myocardial infarction	692 (1)	87 (1)	558 (1)	293 (1)	0.91
Dyslipidemia	31 512 (49)	4028 (50)	29 750 (48)	9951 (44)	0.01
Hypertension	38 624 (60)	4687 (58)	36 434 (59)	12 674 (56)	0.02
Arrhythmia	2899 (4)	363 (5)	2516 (4)	1030 (5)	0.87
Valve disease	1509 (2)	204 (3)	1357 (2)	525 (2)	0.25
History of diabetes complications at index date	5551 (9)	827 (10)	4831 (8)	2268 (10)	<0.001
Estimated glomerular filtration rate categories (mL/min):					
<30	110 (0.2)	22 (0.3)	25 (0.04)	74 (0.3)	
30 to <60	3438 (5)	416 (5)	2775 (4)	1307 (6)	<0.001
60 to <90	37 006 (57)	4237 (53)	35 496 (57)	11 361 (51)	
≥90	24 152 (37)	3357 (42)	23 683 (38)	9728 (43)	
Albuminuria (≥5 g/dL)	1969 (3)	289 (4)	1901 (3)	728 (3)	<0.01
Mean (SD) total cholesterol (mg/dL)	197 (50)	197 (54)	198 (51)	203 (55)	0.90
Mean (SD) triglycerides (mg/dL)	220 (292)	235 (316)	222 (300)	249 (355)	<0.001
Mean (SD) HDL cholesterol (mg/dL)	45 (12)	44 (12)	44 (12)	44 (12)	<0.001
Mean (SD) LDL cholesterol (mg/dL)	114 (37)	112 (36)	114 (36)	116 (39)	0.17
Mean (SD) HbA_{1c} (%)	7.5 (1.8)	8.0 (2.0)	7.6 (1.7)	8.3 (2.1)	<0.001
Mean (SD) hemoglobin (g/dL)	14.3 (1.5)	14.5 (1.5)	14.3 (1.5)	14.4 (1.6)	<0.001
Drug use					
Any metformin use	55 124 (85)	6855 (85)	61 979 (100)	16 825 (75)	0.71
Any sulfonylurea use	19 493 (30)	2977 (37)	16 825 (27)	22 470 (100)	<0.001
Any thiazolidinedione use	16 941 (26)	2833 (35)	14 680 (24)	6859 (31)	<0.001
Any other oral antidiabetic agent use	1189 (2)	321 (4)	1031 (2)	558 (2)	<0.001
Any insulin use	2812 (4)	742 (9)	3034 (5)	2074 (9)	<0.001
ACE inhibitor/ARB/renin inhibitor	24 008 (37)	2959 (37)	22 736 (37)	7175 (32)	0.65
Statin	20 330 (31)	2300 (29)	18 964 (31)	5308 (24)	<0.001
β blocker	13 644 (21)	1566 (20)	12 552 (20)	4366 (19)	0.001

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Table 1 (continued)

Characteristics	No sitagliptin exposure (n=64 706)	Exposed to sitagliptin (n=8032)	Exposed to metformin (n=61 979)	Exposed to sulfonylurea (n=22 470)	P value*
Dihydro calcium channel blocker	7526 (12)	835 (10)	6908 (11)	2463 (11)	0.001
Non-dihydro calcium channel blocker	2501 (4)	261 (3)	2251 (4)	791 (4)	<0.01
Nitrates	1706 (3)	178 (2)	1463 (2)	567 (3)	0.03
Diuretics	11 409 (18)	1209 (15)	10 499 (17)	3482 (16)	<0.001
Anticoagulants	1253 (2)	161 (2)	1068 (2)	419 (2)	0.68
Antiplatelet agents	2244 (3)	269 (3)	1951 (3)	694 (3)	0.58
Healthcare use					
Inpatient hospital admissions in year before index date:					
0	59 479 (92)	7517 (94)	57 796 (93)	20 292 (90)	
1	4285 (7)	453 (6)	3702 (6)	1885 (8)	<0.001
≥2	672 (1)	62 (1)	481 (1)	293 (1)	
Frailty condition	2013 (3)	253 (3)	1940 (3)	692 (3)	0.85
Chronic conditions before index date:					
≤1	21 490 (33)	2570 (32)	21 214 (34)	8027 (36)	0.03
2	13 245 (20)	1566 (19)	12 791 (21)	4502 (20)	
≥3	29 971 (46)	3897 (49)	27 974 (45)	9940 (44)	
Mean (SD) drug possession ratio for diabetes related drugs	0.69 (0.64)	0.75 (0.55)	0.69 (0.63)	0.71 (0.40)	<0.001

Drug columns are not mutually exclusive.

ACE=angiotensin converting enzyme; ARB=angiotensin receptor blocker; HbA_{1c}=glycated hemoglobin; HDL=high density lipoprotein; LDL=low density lipoprotein.

*No sitagliptin exposure compared with exposed to sitagliptin.

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Table 2| **Outcomes according to antidiabetic drug exposure**

Agent*	Time at risk (person years)	Events—No (%)	Unadjusted hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)†	P value
All cause hospital admission or all cause mortality					
Any sitagliptin use	9360	803 (10)	1.01 (0.94 to 1.09)	0.98 (0.91 to 1.06)	0.63
Any metformin use	93 002	7 995 (13)	0.83 (0.81 to 0.86)	0.88 (0.85 to 0.91)	<0.001
Any sulfonylurea use	30 456	3 501 (15)	1.35 (1.30 to 1.40)	1.31 (1.26 to 1.37)	<0.001
Any thiazolidinedione use	28 853	2 477 (13)	0.94 (0.90 to 0.98)	0.98 (0.94 to 1.02)	0.35
Other antidiabetic drug use‡	1396	182 (12)	1.33 (1.15 to 1.54)	1.21 (1.05 to 1.41)	0.01
Any insulin use	3825	679 (20)	2.08 (1.93 to 2.25)	1.89 (1.75 to 2.05)	<0.001
All cause mortality					
Any sitagliptin use	11 307	32 (0.4)	1.02 (0.71 to 1.46)	1.14 (0.79 to 1.65)	0.47
Any metformin use	105 400	172 (0.3)	0.41 (0.34 to 0.49)	0.78 (0.64 to 0.97)	0.02
Any sulfonylurea use	36 405	137 (0.6)	1.49 (1.23 to 1.82)	1.53 (1.24 to 1.87)	<0.001
Any thiazolidinedione use	33057	68 (0.3)	0.70 (0.54 to 0.90)	0.72 (0.55 to 0.93)	0.01
Other antidiabetic drug use‡	1713	17 (1)	2.88 (1.77 to 4.70)	3.29 (2.01 to 5.39)	<0.001
Any insulin use	5801	67 (1)	3.66 (2.82 to 4.76)	3.42 (2.61 to 4.48)	<0.001
All cause hospital admission					
Any sitagliptin use	9360	797 (10)	1.01 (0.94 to 1.09)	0.98 (0.91 to 1.06)	0.60
Any metformin use	93 002	7 942 (13)	0.83 (0.81 to 0.86)	0.88 (0.84 to 0.91)	<0.001
Any sulfonylurea use	30 456	3 478 (15)	1.35 (1.30 to 1.40)	1.31 (1.26 to 1.37)	<0.001
Any thiazolidinedione use	28 853	2 463 (12)	0.94 (0.90 to 0.98)	0.98 (0.94 to 1.03)	0.39
Other antidiabetic drug use‡	1396	180 (12)	1.32 (1.14 to 1.53)	1.21 (1.04 to 1.40)	0.01
Any insulin use	3825	673 (19)	2.08 (1.92 to 2.25)	1.89 (1.74 to 2.04)	<0.001
Cardiovascular related hospital admission					
Any sitagliptin use	10 920	156 (2)	1.05 (0.89 to 1.23)	0.90 (0.77 to 1.07)	0.23
Any metformin use	103 371	1 315 (2)	0.71 (0.65 to 0.76)	0.79 (0.73 to 0.87)	<0.001
Any sulfonylurea use	35 098	739 (3)	1.59 (1.46 to 1.73)	1.32 (1.20 to 1.44)	<0.001
Any thiazolidinedione use	32 325	488 (2)	1.03 (0.93 to 1.13)	1.03 (0.93 to 1.14)	0.55
Other antidiabetic drug use‡	1629	48 (3)	1.77 (1.33 to 2.36)	1.38 (1.03 to 1.84)	0.03
Any insulin use	5320	193 (4)	2.53 (2.18 to 2.94)	2.15 (1.85 to 2.51)	<0.001
Cardiovascular related hospital admission or all cause mortality					
Any sitagliptin use	10 920	178 (2)	1.04 (0.90 to 1.22)	0.92 (0.79 to 1.07)	0.29
Any metformin use	103 371	1 443 (2)	0.67 (0.62 to 0.72)	0.78 (0.72 to 0.84)	<0.001
Any sulfonylurea use	35 098	825 (4)	1.55 (1.43 to 1.68)	1.32 (1.21 to 1.44)	<0.001
Any thiazolidinedione use	32 325	538 (3)	0.99 (0.90 to 1.09)	0.98 (0.90 to 1.08)	0.74
Other antidiabetic drug use‡	1629	57 (3)	1.84 (1.41 to 2.39)	1.49 (1.14 to 1.94)	0.003
Any insulin use	5320	232 (5)	2.66 (2.32 to 3.04)	2.32 (2.02 to 2.67)	<0.001

*Reference category for each agent is "no exposure to that agent" (for example, sitagliptin use *v* no sitagliptin use).

†Time varying Cox proportional hazards models adjusted for age, sex, socioeconomic status, clinical laboratory data (glycated hemoglobin, high density lipoprotein cholesterol, low density lipoprotein cholesterol, triglycerides, estimated glomerular filtration rate, albuminuria, hemoglobin concentrations), prescription drugs, Johns Hopkins adjusted clinical groups score, expanded diagnosis cluster for diabetes, adherence to glucose lowering treatments, total number of hospital admissions in year before index date, total number of chronic conditions, medically frail condition marker, and time varying propensity score.

‡Acarbose, meglitinides, pramlintide.

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Figures

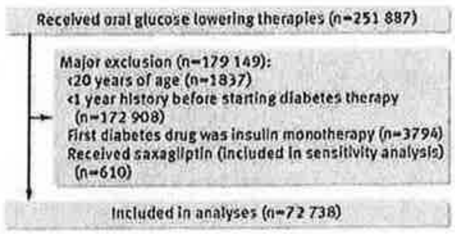

Fig 1 Major exclusions from study

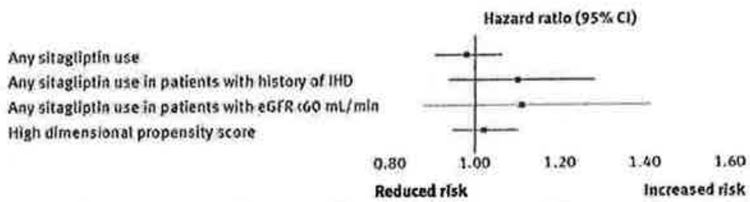

Fig 2 Adjusted hazard ratios and 95% confidence intervals for the outcome of all cause hospital admission or all cause death according to sitagliptin exposure (compared with no sitagliptin use in time varying Cox proportional hazards analysis adjusted for covariates in footnote to table 2). eGFR=estimated glomerular filtration rate; IHD=ischemic heart disease

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EXHIBIT 17

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

25 July 2013
EMA/474117/2013

Assessment report for GLP-1 based therapies

Review under Article 5(3) of **Regulation (EC) No 726/2004**

Procedure no: EMEA/H/A-5(3)/1369

Assessment Report as adopted by the CHMP with all information of a commercially confidential nature deleted.

7 Westferry Circus • Canary Wharf • London E14 4HB • United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

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Table of contents

1. Background information on the procedure

The European Medicines Agency (EMA) was made aware of findings by a group of academic researchers suggesting an increased risk of pancreatitis and cellular changes in patients treated for type 2 diabetes mellitus (T2DM) with GLP-1 based therapies (glucagon-like peptide 1 (GLP-1) agonists and dipeptidylpeptidase-4 (DPP-4) inhibitors)[1]. The findings resulted from the histological examination of 34 pancreata obtained from brain dead organ donors. The pancreata of eight individuals with T2DM who were treated with sitagliptin (n = 7) or exenatide (n = 1) for a year or more were compared to 12 pancreata from individuals with T2DM treated with other therapies and 14 pancreata from non-diabetic individuals. The investigators described a number of findings in the pancreata of the T2DM individuals treated with GLP-1 based therapies which could implicate an association of the treatment with increased risk of pancreatitis and neoplasms.

It was noted that the current product information of all centrally authorised GLP-1 based therapies contains warnings about pancreatitis and that pancreatitis is listed as a reported event. In addition, the incidence rates of pancreatitis and the potential occurrence of pancreatic cancer for authorised GLP-1 based products is being investigated as part of several ongoing studies. However, in view of the new evidence, the Committee for Medicinal Products for Human Use (CHMP) was requested to investigate the emerging data and to give an opinion, under Article 5(3) of Regulation (EC) 726/2004, on the potential impact on centrally authorised GLP-1 agonists and DPP-4 inhibitors products, in consultation with the Pharmacovigilance Risk Assessment Committee (PRAC). In case concerns are identified, the Committees are to indicate whether these should be further investigated at Community level.

2. Scientific discussion

2.1 Introduction

Glucagon-like peptide 1 based therapies are approved for the treatment of patients with type 2 diabetes. These therapies include GLP-1 receptor agonists (exenatide, liraglutide and lixisenatide) and DPP-4 inhibitors (sitagliptin, vildagliptin, saxagliptin and linagliptin) which, albeit in different ways, increase the exposure to GLP-1.

Glucagon-like peptide 1 is a gut hormone secreted by the intestinal epithelial endocrine L-cells as a response to the presence of nutrients in the lumen of the small intestine. Once in the circulation, GLP-1 has a half-life of one to two minutes, due to rapid degradation by the enzyme dipeptidyl peptidase-4 (DPP-4). Due to the short half-life, GLP-1 analogues, resistant to the action of DPP-4, and DPP-4 inhibitors have been developed. The mechanism of these products is to increase the exposure to incretin hormones (mainly GLP-1) which leads to a glucose dependent stimulation of alpha and beta cells. The main actions of GLP-1 are to stimulate insulin secretion (i.e., to act as an incretin hormone) and to inhibit glucagon secretion (the normal glucagon response to hypoglycaemia is not impaired), thereby contributing to limit postprandial glucose excursions. It also inhibits gastrointestinal motility and secretion and thus acts as an enterogastrone and part of the "ileal brake" mechanism. Glucagon-like peptide 1 also appears to be a physiological regulator of appetite and food intake. A number of additional sites with GLP-1 receptors have been discovered including the heart and the nervous system. There are studies supporting that GLP-1 can regulate signaling pathways coupled to cell proliferation and apoptosis.

[1] *Butler et al*, Marked Expansion of Exocrine and Endocrine Pancreas With Incretin Therapy in Humans With Increased Exocrine Pancreas Dysplasia and the Potential for Glucagon-Producing Neuroendocrine Tumors; Diabetes. 2013 Jul; 62(7):2595-604.

The efficacy of GLP-1 receptor agonists and DPP-4 inhibitors has been demonstrated. In terms of safety, the most common adverse events seen in clinical trials with GLP-1 receptor agonists are of gastrointestinal character; mainly nausea, vomiting and diarrhoea. However, the incidence diminishes over time. Other identified risks include pancreatitis, immunogenicity, acute renal failure and rapid weight loss. Identified and potential risks with DPP-4 inhibitors include hypoglycaemia, hypersensitivity, gastrointestinal disorders, pancreatitis, skin disorders, transaminase elevation and infections.

The current review was initiated further to the findings by a group of academic researchers suggesting an increased risk of pancreatitis and cellular changes in patients treated for T2DM with GLP-1 based therapies (*Butler et al*, 2013). The CHMP considered the recently published article on this matter and a review of available pre-clinical and clinical information with respect to pancreatic safety was undertaken. The PRAC was consulted, as applicable. The outcome of an ad-hoc expert meeting held was also considered. Only relevant information for the discussion is presented hereinafter.

2.2 Butler et al (2013)

A summary of the main findings of the publication by *Butler et al*, 2013 is described hereinafter.

<u>Study design and methods</u>

The study examined pancreata from organ donors with type 2 diabetes mellitus (DM) treated by incretin therapy (n=8) or other therapy (n=12) and non-diabetic controls (n=14). All pancreata were procured from brain dead organ donors by the JDRF Network for Pancreatic Organ Donors with Diabetes (nPOD) coordinated through the University of Florida in Gainesville, Florida. The eight subjects who received incretin therapy had been treated for a year or more (seven treated with the DPP-4 inhibitor sitagliptin and 1 with the GLP-1 agonist exenatide).

The subjects characteristics, including age, duration of disease, body mass index (BMI), treatments received and captured cause of death are listed below.

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Table 1 Clinical characteristics of brain-dead organ donors (as presented in the publication)

Case	Age (years)	Duration of DM (years)	Sex	BMI (kg/m^2)	Treatments	Cause of death
DM-I						
6157	74	1	F	39	Januvia	ICH/stroke
6185	46	15	M	41	Januvia, metformin	Anoxia
6186	68	5	M	21	Januvia, metformin	ICH/stroke
6189	49	26	F	36	Byetta, metformin, glipizide	Stroke
6199	53	20	M	30	Januvia, insulin pen	ICH/stroke
6194	47	13	M	24	Humulin, NovoLog, Januvia	ICH/stroke
6203	68	5	M	33	Januvia, metformin	Stroke
6206	59	10	M	42	Januvia, metformin	Stroke
Mean (SEM)	58 (4)	12 (3)		33 (3)		
DM						
6028	33	17	M	30	Insulin	Gunshot wound to head
6059	18	0.3	F	39	None	Cardiovascular
6108	57	2	M	30	Metformin	ICH/stroke
6110	20	0.2	F	40	None	ICH/stroke, DKA
6109	48	—	F	33	None	ICH/stroke, DKA
6114	42	2	M	31	Metformin, noncompliant	Asphyxiation
6124	62	3	M	34	Metformin	ICH/stroke
6127	44	10	F	30	Insulin	ICH/stroke
6133	45	20	F	40	Insulin	Cardiovascular
6139	37	1.5	F	45	None	Seizure
6142	29	14	F	34	None	Bacterial meningitis
6149	39	20	F	29	Insulin	ICH/stroke
Mean (SEM)	40 (4)	8 (3)		36 (2)		
ND						
6009	45		M	31		Anoxia
6015	39		F	32		Anoxia
6012	64		F	31		Cerebrovascular/stroke
6016	42		M	31		Cerebrovascular/stroke
6019	68		F	24		Head trauma
6020	60		M	30		Cerebrovascular/stroke
6022	75		M	31		Cerebrovascular/stroke
6034	32		F	25		Head trauma
6060	24		M	33		Head trauma
6097	43		F	36		Cerebrovascular/stroke
6099	14		M	30		Head trauma
6102	45		F	35		Cerebrovascular/stroke
6158	40		M	30		Head trauma
6165	45		F	25		Cerebrovascular/stroke
Mean (SEM)	45 (5)			30 (1)		

DKA, diabetic ketoacidosis; F, female; ICH, intracerebral hemorrhage; M, male.

In terms of pancreas fixation, embedding and sectioning, the authors described the preparation procedure for pancreata recovered from cadaveric organ donors. Immunostaining was performed in two locations and included: 1) the deparaffinization of serial sections and incubation with primary antibodies to Ki67 and insulin, or CD3 and glucagon with antibody localization visualized with peroxidase-DAB (3, 3'-diaminobenzidine) and alkaline phosphatase-Fast Red polymer systems; 2) staining for Ki67, insulin and Alcian blue by immunohistochemistry and Ki67 and glucagon by immunohistochemistry. A section of pancreas from each of the DM cases treated with incretin therapy and a subset of DM not treated with incretin therapy (5 cases) and non-diabetic cases (6 cases) were stained for insulin and glucagon by immunofluorescence, and additional sections for glucagon, insulin, cytokeratin and DAPI (4',6-diamidino-2-phenylindole).

The stained slides or sections of pancreas were scanned. The morphometric analysis was either through estimating the proportion of insulin and glucagon stained area compared to total tissue area defined by hematoxylin counterstain using an algorithm or measuring the total area of the tissue. Full cross-sections of the pancreas head, body and tail were evaluated for pancreatic intraepithelial neoplasia (PanIN) by a gastrointestinal pathologist blinded to clinical information. The number of PanIN lesions and grade were established per lobular unit and then computed per unit area of pancreas. Using certain stained sections, 100 islets were analysed per section to determine the frequency of Ki67 in the alpha and beta cells of islets and in the non-alpha and non-beta cell compartment of those islets.

A total of 475 alpha cells and 475 beta cells were evaluated. The percentage of beta and alpha cells within pancreatic ducts was determined and the methodology used was described by the authors.

Results

According to the publication, pancreatic mass was increased ($p<0.05$) by approximately 40% in DM patients treated with incretin therapy compared to that observed in subjects with DM and not treated with these medicinal products.

The beta cell mass was decreased by 55% in DM patients not on incretin therapy in comparison to non-diabetic controls (0.29±0.08 *vs.* 0.60±0.10G; $p<0.05$), whilst an increase, mostly on beta cell numbers rather than beta cell size, was noted in incretin treated DM patients compared to the DM group (1.81±0.56 *vs.* 0.29±0.08G, $p<0.01$) and to non-diabetic controls (1.81±0.56 *vs.* 0.60±0.10 G, $p<0.05$).

The pancreatic fractional area immunostained for glucagon was increased in individuals with DM treated with incretin therapy in comparison with those with DM on other therapy (1.65±0.39 *vs.* 0.57±0.12%, $p<0.0001$), as well as compared to non-diabetic controls (1.65±0.39 *vs.* 0.52±0.08%, $p<0.0001$). The glucagon mass pattern was also increased in DM individuals treated with incretin therapy compared to those with DM not treated with these medicines (2.08±0.75 *vs.* 0.45±0.10 G, DM-I vs. DM, $p<0.01$). As for beta cells, the increase in alpha cell mass was mostly due to an increase in the number of alpha cells.

The authors reported a subset of enlarged and peculiar shaped islets, as well as increased numbers of endocrine cells in association with duct structures in DM subjects treated with incretin therapy. Insulin immunoreactive cells were found in individuals from all three groups with no detectable increase between groups regardless of incretin therapy. However, the percentage of cells immunoreactive for glucagon in ducts was increased in DM subjects with prior incretin therapy versus DM subjects not treated with incretin therapy (2.8±0.9 *vs.* 0.5±0.2%, p=<0.05). It was noted that the increase in glucagon immunoreactive cells with incretin treatment were mostly observed in the periductal areas whilst the increased numbers of insulin immunoreactive cells with incretin therapy were located in more remote areas from these periductal endocrine complexes.

Alpha cell hyperplasia was reported in one subjected with DM and treated with exenatide. In one individual with DM treated with sitagliptin, an alpha cell/glucagon producing neuroendocrine tumor was identified in the body of the pancreas. Glucagon-producing microadenomas were also detected in the same case and two other incretin treated cases, while hyperplastic islets with predominant glucagon staining were noted in seven of eight of the incretin treated cases. No neuroendocrine tumors or glucagon-producing microadenomas were detected in non-diabetic controls or DM subjects not treated with incretin therapy. The authors indicated that an inspection of pancreatic sections immunostained with either insulin or glucagon from individuals with DM treated with incretin therapy seemed to suggest that several cells within these islets were immunoreactive for both hormones. The percentage of insulin positive cells in incretin treated individuals that were also glucagon immunoreactive were increased when compared to those with DM not treated with incretin therapy (16.8±5.0 *vs.* 3.2±1.4%, $p<0.05$). There was also an increase in double immunoreactive positive cells in individuals with DM not treated with incretin therapy when compared to non-diabetic controls (3.2±1.4 *vs.* 0.4±0.1%, $p<0.05$).

The frequency of Ki67 positive nuclei in islet endocrine cells was extremely rare (all less than 0.01 cells per islet section), with no significant differences between the three groups studied.

Finally, it was noted that the increased pancreatic mass in DM-incretin therapy was accompanied by increased whole pancreas cell proliferation (0.25±0.03 *vs.* 0.12±0.01%, DM-I *vs.* DM, $p<0.0001$) and an increase in the presence of pancreatic intraepithelial neoplasia (PanINs) (11.9±2.6 *vs.* 4.9±1.7, DM-I *vs.* DM, PanINs/mm2 x 103, $p<0.01$). Inspection of pancreas sections in incretin treated individuals revealed small foci of increased Ki67 immunostaining in and around ducts and sometimes in areas of exocrine dysplasia.

2.3 Preclinical and clinical data on pancreatic safety

Preclinical and clinical information previously available was considered by the CHMP, with a focus on pancreatitis and/or pancreatic cancer. Current pharmacovigilance activities and ongoing studies aiming to collect information on pancreatic events were also considered. A summary for GLP-1 agonists (exenatide, liraglutide and lixisenatide) and DPP-4 inhibitors (sitagliptin, vildagliptin, saxagliptin and linagliptin) is presented below.

Exenatide

In vitro and animal pharmacology studies with exenatide have shown an increase in beta-cell mass following treatment. No adverse effects on the pancreas of healthy animals were observed in any of the toxicology studies included in the initial marketing authorisation application. However, further studies performed by academic groups have demonstrated a potential for other effects in the pancreas. *Gier et al,* 2012 Diabetes 61:1250 showed an increase in pancreatic duct glands in rats treated with exenatide. They also showed that this effect in an oncogene-expressing transgenic mouse could contribute to dysplasia and/or pancreatitis. The relevance of these findings for clinical safety is uncertain. In the non-human primate studies, there was a mild pancreatic hypercellularity in monkeys treated for 3 and 9 months. The effect was only seen at the highest dose, representing an exposure margin to clinical exposure of approximately 1000-fold. There were no suggestions of toxicologically important changes from histopathology. Given that increased beta-cell mass was considered a potentially important mechanism for the adventitious effects of GLP-1 receptor agonists, the mild pancreatic hypercellularity in monkeys was not considered a concern. Moreover, in the carcinogenicity studies in mice and rats, there was no evidence for pancreatic neoplasia.

In the clinical setting, safety data from the clinical trial programm did not suggest an increased risk of pancreatitis with exenatide twice a day (BID) compared to other drugs. However, at the time of approval, spontaneous cases of pancreatitis had been reported in other markets in which the products had already been introduced. The product information therefore contains wording with regards to pancreatitis as a warning and a listed undesirable effect. In clinical trials two cases of pancreatic cancer have been reported. In the Integrated Completed Studies Database supporting the exenatide once weekly (QW) submission, there were three cases of acute pancreatitis (one in a subject receiving exenatide QW and two in subjects receiving pioglitazone). No case of pancreatic neoplasm was reported in the database.

Results from three retrospective studies evaluating the risk of pancreatitis as well as data from a registry with respect to risk of pancreatic neoplasm concluded that the studies did not show a risk difference between current or recent use of exenatide compared to other oral antidiabetic drugs. However, it was also concluded that the evidence needs to be weighed with caution, due to the nature of the data with high risk of residual confounding. However, due to the low number of pancreatic neoplasms, no firm conclusions can be drawn.

In addition to routine pharmacovigilance, ongoing post-marketing initiatives include a trial on the evaluation of cardiovascular outcomes with linagliptin therapy which will also collect information with regards to pancreatic events. Furthermore, observational studies and prescription event monitoring studies are also ongoing.

Liraglutide

Repeat-dose toxicity studies were conducted in CD-1 mice, Sprague Dawley rats and Cynomolgus monkeys. In addition, long-term carcinogenicity studies were conducted in mice and rats. An increased pancreatic weight was observed in the mid and high dose groups of Cynomolgus monkeys at 52 weeks treatment (study duration up to 87 weeks). The weight increase was shown to be related to a balanced increase in exocrine duct and acini mass, however the duct/acinar weight ratio was constant between the control and high dose animals. Normal histological morphology of the pancreas was seen in all studies, no clinical or biochemical changes were seen in any of the non-human studies and there was no histopathology indicative of inflammation. In addition, no macroscopic changes were observed in the 87 week repeat dose toxicity study in Cynomolgus monkeys, therefore the findings at week 52 do not suggest a safety concern for humans with respect to treatment related pancreatitis. Overall the non-clinical data do not indicate that liraglutide treatment is associated with adverse effects on the endocrine and exocrine pancreas. A post marketing authorisation study performed in Zucker diabetic fatty (ZDF) rats also showed that liraglutide treatment was not associated with pancreatitis and no increased exocrine cell mass or exocrine cell proliferation was observed.

In terms of clinical data, the reporting rates of acute pancreatitis and pancreatitis in Phase IIIa trials was 1.6/1,000 subject years of exposure (SYE) for liraglutide and 1.4/1,000 SYE for oral antidiabetic drugs. One death due to pancreatic carcinoma was also identified and considered as not related to treatment. Cases of pancreatitis and neoplasms are followed up in periodic safety update reports. Based on evidence from clinical trials and the post-marketing phase, the product information has been kept up to date and contains wording with regards to pancreatitis as a warning and a listed undesirable effect.

In addition to routine pharmacovigilance, ongoing post-marketing initiatives include a trial on the evaluation of cardiovascular outcomes with liraglutide therapy which will also collect information with regards to pancreatic events. Observational studies are also ongoing.

Lixisenatide

Repeat-dose toxicity studies were conducted in mice, rats and dogs. The potential effect of lixisenatide on the absolute and relative pancreas weights was not assessed. In two-year carcinogenicity studies performed in mice and rats, some microscopic findings were reported. When histopathological changes were detected in the pancreas (islet cells hyperplasia, islet cells adenoma, acinar cells hyperplasia) they occurred at high exposure levels compared to expected active exposure in clinical practice, in a small number of animals and with a low degree of severity. No gender- or dose-effect relationships were observed. With regards to the incidence of islet cell adenoma/carcinoma seen in rats dosed with lixisenatide, there was no statistically significant difference between these drug-treated rats as compared to the control animals. The microscopic findings were not considered to be indicative of a high clinical safety risk.

In the clinical setting, adverse events specific to pancreatitis were reported in phase II/III studies in nine patients in the lixisenatide group (0.3%) compared to two in the placebo group (0.1%). However, when the events of acute pancreatitis and pancreatitis were confirmed, by either gastroenterological consultation or positive imaging studies, the incidence was found to be similar between treatment groups. Pancreatic carcinoma was reported in three (<0.1%) lixisenatide patients and one (<0.1%) patient in the comparator group (exenatide arm).

Based on evidence from clinical trials, the product information contains wording with regards to pancreatitis as a warning.

In addition to routine pharmacovigilance, ongoing post-marketing initiatives include a trial on the evaluation of cardiovascular outcomes with lixisenatide therapy which will also collect information with regards to pancreatic events. A retrospective database study and a patient registry are planned to monitor occurrences of events of interest, e.g. pancreatitis and pancreatic cancer.

Sitagliptin

In *in vivo* studies, including repeated-dose studies in mice, rats, dogs and monkeys and carcinogenicity studies in mice and rats, no adverse effects on the pancreas were observed. It has also been shown that sitagliptin is not a genotoxic compound *in vitro* and *in vivo*. In non-human primates, potential effects on the pancreas were evaluated in a three month repeated-dose toxicity study. The histopathology data on the pancreas showed no concern. In literature, sitagliptin was observed to cause ductal proliferation and metaplasia in a transgene model of the diabetic rat (*Matveyenko et al* 2009 Diabetes 58:1604), however data from HIP (human islet amyloid polypeptide transgenic) mice and ZDF (Zucker diabetic fatty) rats support the beneficial effect of sitagliptin on beta-cell function, primarily mediated by an improved beta-cell preservation, e.g. by reducing beta-cell death (apoptosis) rather than by expanding of beta-cell mass by cell proliferation of the pancreatic duct. In these studies, cell proliferation of pancreatic duct cells, an important risk factor for the development of pancreatitis and pancreatic cancer, was not increased by sitagliptin as compared to metformin.

Two cases of pancreatitis and two cases of pancreatic carcinoma were reported in the initial clinical trials supporting the marketing authorisation. The data were considered insufficient to draw conclusions. In another trial one case of pancreatic cancer was also reported. Pancreatitis and pancreatic cancer have been reported in the post-marketing setting. With regards to pancreatic cancer, the data do not indicate a true association. A cumulative review of cases has been undertaken and the majority (19 out of 29) had a time to onset < 6 months, a period considered too short to suggest a causal relationship with sitagliptin. Further post-marketing cases did not show any change of pattern or increase in incidence.

Based on evidence from clinical trials and the post-marketing phase, the product information has been kept up to date and contains wording with regards to pancreatitis as a warning and a listed undesirable effect.

In addition to routine pharmacovigilance, ongoing post-marketing initiatives include a trial on the evaluation of cardiovascular outcomes with sitagliptin therapy which will also collect information with regards to pancreatic events.

Saxagliptin

All repeat dose and carcinogenicity studies were performed in non-diabetic animals. No findings indicative of pre-neoplastic lesions or proliferative effects were observed in repeat dose toxicity studies in mouse, rat, dog or monkey at plasma exposure levels adequately above human exposure levels at maximal therapeutic dose. Saxagliptin was non-genotoxic *in vitro* and *in vivo*. At plasma exposure levels adequately above human exposure levels at maximal therapeutic dose, saxagliptin did not lead to pancreatic hyperplasia or neoplasia.

In the clinical setting, there was no evidence for any causal relation between treatment with saxagliptin and pancreatic neoplasms in data from phase IIb and III studies. Four cases of pancreatitis at least possibly related to treatment with saxagliptin were reported. Pancreatitis has also been reported in the post marketing phase. A total of eight cases of pancreatic cancer and two cases of pancreas neoplasm have been reported. Duration of treatment with saxagliptin was known in six cases, ranging from 4-18 months. The short time to event, not expected in drug-induced malignancies, and a lack of sufficient background information makes causality assessment difficult.

Based on evidence from clinical trials and the post-marketing phase, the product information has been kept up to date and contains wording with regards to pancreatitis as a warning and a listed undesirable effect.
In addition to routine pharmacovigilance, ongoing post-marketing initiatives include a trial on the evaluation of cardiovascular outcomes with saxagliptin therapy which will also collect information with regards to pancreatic events.

Vildagliptin

The influence of vildagliptin on beta-cell regulation was examined in neonatal rats and in streptozotocin (STZ)-induced diabetic mice. Vildagliptin markedly increased replication (>8-fold increase) and inhibited apoptosis (by 65%) on day 7 of treatment. This resulted in a significant increase in beta-cell mass on day 21 (24-h after final dose), which was maintained on day 33 (12-d after final dose). There was no apparent effect of treatment on duct-associated beta-cells (an index of neogenesis) or on glucagon staining in neonatal rats. The vildagliptin inhibition of apoptosis was coherent with the results reported by *Hamamoto S et al*, 2013 in obese diabetic KK-Ay mice, where the authors concluded that in the mouse model used vildagliptin increases beta-cell mass by suppressing cell apoptosis and oxidative stress and by enhancing cell proliferation and differentiation. An effect on the alpha cell mass was not observed. Vildagliptin did not shown genotoxic potential *in vitro* and *in vivo*. The carcinogenic potential was investigated in rats and mice in 2-year carcinogenicity studies. In the rat survival was not affected by treatment. An increased incidence of hemangiosarcoma in male mice treated at ≥ 250 mg/kg/day and in female mice at 1000 mg/kg/day (exposure ratio of 15 at the no observed adverse effect level [NOAEL] of 100 mg/kg/day) was reported, but the findings were found to not represent a significant risk to humans.

In the clinical setting, pancreatitis-related adverse events were reported infrequently with similar incidences across all treatment groups in phase II/III clinical trials. Only a very small number of pancreatic cancer events were reported in vildagliptin and comparator groups (three each), translating into 0.032 cases per 100 SYE vs. 0.046 cases per 100 SYE, respectively. Pancreatitis has also been reported in the post marketing phase, with the majority of cases resolving after drug interruption. In terms of pancreatic cancer, in nine of the 15 cases where time to onset was reported, pancreatic cancer occurred within three months after treatment initiation. This short time does not allow consideration of a direct drug induced neoplasm, although a promoting effect of vildagliptin on preexisting lesions cannot be excluded.
Based on evidence from clinical trials and the post-marketing phase, the product information has been kept up to date and contains wording with regards to pancreatitis as a warning and a listed undesirable effect.
In addition to routine pharmacovigilance, ongoing post-marketing initiatives include a long-term observational study to assess various safety outcomes in association with vildagliptin or the fixed-dose combination of vildagliptin plus metformin, including pancreatic events. A multinational observational study to assess the profile of vildagliptin in a real world setting is also ongoing.

Linagliptin

In non-clinical studies pancreatic morphology was investigated in the mouse, rat, dog and monkey. No consistent findings were obtained, neither in respect to pancreatitis nor in respect to proliferation. Linagliptin did not show a genotoxic potential and did not induce carcinogenic effects in the 2-year carcinogenic mouse study, except for a significant increase in malignant lymphomas in females. This was attributed to a high background of lymphomas in mice. Because linagliptin is not genotoxic and lymphoid hyperplasia in spleen and thymus was not increased in female mice, it was concluded that this finding was not relevant for humans.

Available clinical data from a large number of patients in placebo-controlled clinical trials showed that the incidence of pancreatitis in the linagliptin group is low (0.22 cases per 100 patient years in the linagliptin group vs. 0.07 per 100 patient years in the placebo group; the difference did not reach statistical significance). Cases of pancreatitis and neoplasms are followed up in periodic safety update reports. No conclusions on pancreas carcinoma can be drawn at present due to the low number of cases reported. Based on evidence from clinical trials and the post-marketing phase, the product information has been kept up to date and contains wording with regards to pancreatitis as a warning and a listed undesirable effect.

In addition to routine pharmacovigilance, ongoing post-marketing initiatives include a trial on the evaluation of cardiovascular outcomes with linagliptin therapy which will also collect information with regards to pancreatic events.

2.4 Other initiatives

Ad-hoc expert meeting

An ad-hoc expert meeting was convened on 10 July 2013 on a number of aspects of the *Butler et al* 2013 publication and to inform the CHMP.

Overall the experts considered that there were a high number of methodological issues, confounding factors and potential sources of bias observed in the *Butler et al* 2013 publication and that these precluded any meaningful conclusions to establish a link between the use of GLP-1 based therapies and morphological changes of the pancreas indicating an increased risk of pancreatic malignancies.

With regards to patient selection, the experts considered that the three groups compared in this study (T2DM patients on GLP-1 based therapy, T2DM patients on other or no therapy and the non-diabetic patient controls) were very much mismatched, in particular with regard to age, sex, and to some extent body mass, with all three parameters having variable impact on pancreas findings. Information on previous treatments and the duration of these treatments was also considered to be lacking The mean age of the GLP-1 treated group was 58 years of age, which is significantly higher than the mean age of the non-GLP-1 treated group (40 years) or the control group (45 years), partly due to a number of very young individuals included in the two control groups. The experts agreed that the groups should have been better matched with regard to age through appropriate selection of cases from the nPOD tissue bank. The experts also pointed out that the two diabetic patient groups were mismatched in terms of gender, with the GLP-1 treated group being composed of two females and six males, while the non-GLP-1 group consisting of eight females and four males.

The presence of autoantibody titres (insulin and GAD) in one third of the individuals, a history of diabetic ketoacidosis in one fourth of the T2DM control group and the young age of some individuals in the control groups (18 and 20 in the non-GLP-1 group and 24 and 14 in the n-T2DM group) raised concerns of a possible misclassification of at least some of tnese patients as T2DM instead of type 1 diabetes mellitus (T1DM). However, the possibility that all these individuals were indeed T2DM patients was acknowledged, as autoantibodies can be non-specific and ketoacidosis may be observed in some T2DM patients. The experts were of the view that clinical data, including detailed treatment history of the patients, was lacking, although the difficulty in obtaining this data from nPOD due to personal data protection issues was acknowledged.

No concerns were raised regarding the fixation or the embedding and the preservation of the tissues was considered good. However, the experts considered that the substandard staining, the lack of rigorous analysis and the unclear description of the methodological approach raised concerns which could have a major impact on the validity of the conclusions reached by the authors. Issues discussed referred to under-stained and over-stained alpha and beta cells, almost identical compartments within the same islet regions staining positively both for insulin and glucagon, and staining of the acinar area and connective tissue. Consideration should have been given to staining for other types of hormones, such as somatostatin. With regard to sectioning, evidence of a systematic sectioning approach ensuring that samples from all three regions was lacking and variations in sectioning methods and sample selection may have led to biased results. Measuring volume instead of area would have been more adequate with regard to estimation of alpha and beta-cell mass.

The experts considered the results identified in the publication with regard to changes in alpha and beta cell mass and in overall pancreatic mass to be inconclusive, given the uncertainty raised by major study deficiencies regarding the patient selection and the morphometric analysis. Pancreatic weight should have been adjusted for the height, weight, age and gender of the individual donor, according to available algorithms. Changes in the fat content of the pancreas (in particular in obese individuals) should have been considered as a cause for differences in pancreatic weight.

Overall, the experts considered that the presented evidence did not support the view that GLP-1 based therapies resulted in histological changes of the pancreas in these individuals indicating an increased risk of pancreatic adenocarcinoma. No reports of clinical symptoms for glucagonoma were available and it was noted that patients with glucagonoma tend to lose weight due to wasting, rather than being obese, as observed in the GLP-1 group (the three individuals in which the glucagon-positive neuroendocrine tumour and microadenomas were observed had BMI values of 39, 41 and 42 respectively). The presence of cells staining positive for glucagon would also not necessarily indicate secretion of glucagon by these cells. Moreover, the reliability of the staining was considered questionable, as mentioned above. It was noted that glucagonomas are rare tumours with an incidence of approximately one in 200.000, and that given the widespread use of GLP-1 based therapies, any increase in the incidence of clinically relevant glucagonomas should have been noticed by now.

A study by *Kimura et al* (1991) reviewing pancreata from 800 consecutive autopsies, identified endocrine tumours (including microadenomas) and islet hyperplasia in 10 percent of adult patients, with most of these lesions staining positive for glucagon. The study also indicated that the detection of such lesions depends heavily on the level of scrutiny and that significantly more tumours are found when larger numbers of slides are examined. In view of the apparent relatively high prevalence of small clinically asymptomatic endocrine tumours in the general population and the lack of information on the screening methodology use in the Butler study, the experts found the true significance of their finding of three cases with one or more clinically asymptomatic (micro)adenomas difficult to evaluate. More detailed histopathological studies on larger patient groups would be necessary to address this issue.

Discussion

Glucagon-like peptide 1 based therapies [GLP-1 receptor agonists (exenatide, liraglutide and lixisenatide) and DPP-4 inhibitors (sitagliptin, vildagliptin, saxagliptin and linagliptin)] are approved for the treatment of patients with type 2 diabetes mellitus (T2DM).

The efficacy of GLP-1 receptor agonists and DPP-4 inhibitors has been demonstrated. In terms of safety, the most common adverse events seen in clinical trials with GLP-1 receptor agonists are of gastrointestinal character; mainly nausea, vomiting and diarrhoea. However, the incidence diminishes over time. Other identified risks include pancreatitis, immunogenicity, acute renal failure and rapid weight loss. Identified and potential risks with DPP-4 inhibitors include hypoglycaemia, hypersensitivity, gastrointestinal disorders, pancreatitis, skin disorders, transaminase elevation and infections.

The current review was initiated further to the findings by a group of academic researchers suggesting an increased risk of pancreatitis and cellular changes in patients treated for type 2 diabetes mellitus with GLP-1 based therapies (Butler et al, 2013). The findings in this study were based on histological examinations of 34 pancreata obtained from brain dead organ donors. The pancreata of 8 individuals with T2DM who were treated with sitagliptin (n = 7) or exenatide (n = 1) for a year or more were compared to 12 pancreata from individuals with T2DM treated with other therapies and 14 pancreata from non-diabetic individuals. In their publication, the investigators describe a number of findings in the pancreata of the T2DM individuals treated with GLP-1 based therapies which could implicate an association of the treatment with increased risk of pancreatitis and neoplasms.

An ad-hoc expert meeting was held on 10 July 2013 to discuss the publication and inform the CHMP opinion. The CHMP considered, taking into account the experts' opinion, that the comparison between patients with DM with and without incretins was complicated by the fact that those without incretins may not have had type 2 diabetes considering that only three of 10 patients were on metformin (the rest no treatment or insulin). Some patients, in particular the four younger patients on insulin may have had type 1 diabetes, which would have impact on the validity of the comparison of DM patients with and without incretins. In addition, there were substantial differences between the diabetes patients with and without incretins with respect to age, gender and duration of diabetes, factors that are likely to have impact on the pancreatic findings. Thus, it cannot be concluded that differences between the groups are due to the treatment with sitagliptin/exenatide.

In the incretin treated group, there was an increased alpha and beta cell area and mass as well as pancreatic mass compared to the other groups. The authors stated that these findings were consistent with prior rodent studies (Matveyenko, Diabetes 2009, Gier Diabetes 2012) that revealed proliferative actions of GLP-1 on the endocrine and exocrine pancreas, but also that previous reports suggest a wide range of change in alpha and beta cell mass (or pancreatic fractional area) in patients with DM (Rahier, 2008, Diabetes Obes Metab, *Henquin*, 2011 Diabetologia,). Therefore there are uncertainties as to the importance of these findings in the context of what could be expected in patients with type 2 diabetes as well as possible clinical implications. Furthermore, as mentioned above, the difference between the groups with respect to age, gender and duration of diabetes preclude meaningful interpretation of the data.

In one individual, a glucagon expressing neuroendocrine tumour was detected. Further, glucagon-expressing microadenomas were found in three patients while hyperplastic islets with predominant glucagon staining were noted in seven of eight of the incretin treated cases. In relation to these findings, as well as the findings of increased alpha and beta cell area and mass, the authors questioned the safety of long term suppression of glucagon secretion and action and refer to available preclinical studies indicating an association between suppressed glucagon secretion or signaling and alpha cell hyperplasia, abnormal alpha cell distribution and predisposition to glucagon expressing neuroendocrine tumours. It is agreed that long term suppression of glucagon represents a non-physiological condition. However, as concluded by the ad hoc expert group, according to literature (Kimura et al, 1991, Digestive disease and sciences, vol 36, No 7), microadenomas can be expected to be found in 10% in the general population. Furthermore, a recent publication by *Drucker et al* (Diabetes online July 1st , 2013), reviewed preclinical studies reporting changes in cell numbers in preclinical studies with DPP-4 inhibitors. One of twenty studies described an increase, six studies reported no change and 13 papers described a reduction in alpha-cell number and/or decreased alpha-cell proliferation. Thus, there seems to be limited support for an alpha-cell promoting effect. Concerning the glucagon expressing tumour, the relevance of this case is questioned considering the lack of clinical data as well as unspecific staining reported in the publication.

The CHMP also noted that there was an increased number of endocrine cells in association with duct structures as well as an increase in the presence of pancreatic intraepithelial neoplasia (PanINs). According to the authors, this was consistent with the prior finding that GLP-1 receptors are expressed not only in the human exocrine pancreas but also in PanINs, and that GLP-1 induces proliferative signaling in human pancreatic duct epithelia cells. According to the expert meeting, PANin 1 and 2 are not considered to be prognostic factors for pancreatic cancer, neither for chronic pancreatitis, and more importantly, the incidence of such findings increase with age.

In addition to the Butler publication, the CHMP also considered other evidence from GLP-1 based therapies with regards to pancreatic events. The GLP 1 receptor is expressed in the pancreas, so some effects on the pancreas upon chronic activation of signaling pathways are to be expected. Studies on normal healthy animals did not show any evidence for toxicological action, but for some of the products and particularly in monkeys, there have been findings on increased weight and hypercellularity of the pancreas. While some data show an increase in beta cells, an expected and potentially advantageous effect in the diabetic patient, these data are not conclusive and an effect also on alpha cells and/or cells in the exocrine pancreas cannot be excluded. Importantly, histological examination of the pancreas did not show any evidence for pathological changes associated with the increased pancreas weight/hypercellularity.

In long-term carcinogenicity studies in mice and rats, the pancreas was not a target organ; no findings on pancreatic neoplasia were observed for any of the products. It is also noted that an extensive analysis of pancreata from mice, rats and non-human primates treated with the GLP-1R analog liraglutide for up to 2 years is published, showing that there was no evidence for treatment-related pancreatitis or pre-neoplastic lesions in any of the studies (*Nyborg et al* 2012, Diabetes 61:1243). The safety studies have been performed in healthy animals, and the interaction of the medicinal product and the underlying disease has not been studied. In the development programs for these products, disease models have been used for pharmacological studies. For some of the products three-month pancreatic toxicity studies in the diabetic ZDF rat have been performed post-approval. In these studies performed with liraglutide (*Vrang et al* 2012 Am J Physiol Endocrinol Metab. 15:E253), exenatide (*Tatarkiewicz et al* 2012 Diabetes Obes Metab. 15:417) and sitagliptin there was no evidence for adverse effects in the pancreas.

Other publications have described potentially adverse effects of treatment. In rats carrying a transgene for human islet amyloid polypeptide, a model for type 2 diabetes, 12 weeks of treatment with sitagliptin resulted in increased pancreatic ductal turnover, ductal metaplasia, and in one rat, pancreatitis (*Matveyenko et al* 2009 Diabetes 58:1604). In another study it was found that in normal rats treated with exenatide for 12 weeks, pancreatic duct glands were expanded. Pancreatic duct glands have been hypothesised to give rise to pancreatic intraepithelial neoplasia (PanIN). In transgenic mice expressing an oncogenic Kras mutant in pancreas, 12 weeks of exenatide treatment increased duct cell replication, increased the formation of dysplastic PanIN lesions, and accelerated the development of chronic pancreatitis (*Gier et al* 2012 Diabetes 61:1250). The relevance of these findings for clinical safety is uncertain.

Nonclinical animal data may aid in determining the causal relationship between GLP-1 based therapy and development of pancreatitis and/or pancreatic cancer by identifying pharmacological mechanisms and biomarkers that can be studied in the clinical setting. If such biomarkers, shown to be directly related to pharmacological activity in the animal studies, could be correlated with pancreatic adverse events in the clinical setting a causal relationship would be strengthened. At this point of time, it is not considered that available non-clinical data support such relationship.

With regards to available clinical data, overall, there have been very few cases of pancreatitis detected in the phase II and phase III studies. Incidence rates were presented for some products ranging between 1.6-2.6 cases per 1000 patient years. For some products (e.g. exenatide, lixisenatide, linagliptin) there was a numerically higher incidence compared to placebo. According to literature data, patients with type 2 diabetes have an almost threefold greater risk of pancreatitis compared to patients without diabetes (*Noel RA*, 2009, *Whitcomb* 2006, *Forsmark CE,* 2007, *Girman CJ*, 2010). The estimated incidence rate for pancreatitis in the diabetes population is 4.2 to 5.6 per 1000 patient years (*Garg et al,* 2010, Diabetes Care 33(11):2349-2354 and *Noel et al.* 2009, Diabetes care 32 (5):834-838). In the post marketing setting, a significant number of pancreatitis cases have been reported and these need to be interpreted cautiously. Cumulative rates of pancreatitis were presented for some products, with a range from 0.1 to 0.9 per 1000 patient years. It should be noted that these numbers come from spontaneous reporting of adverse events and estimations of exposure based on sale figures, respectively, and thus are associated with great uncertainty. For this reason it is recognised that reporting rates cannot be directly compared to the estimated risk in the general population or in the population with T2DM also due to known under reporting. The reporting rates seem to be consistent over time for the products which has been marketed for the longest time (e.g. exenatide BID and vildagliptin). Having said this, severe and also fatal cases have been reported and a causal relationship between treatment and pancreatitis is possible. The CHMP noted that the product information for all products already contains warnings with regards to pancreatitis and this is included in the risk management plans.

Concerning pancreatic cancer, in clinical trials, only single cases have been reported for some products and the duration of exposure was in the majority of the cases too short to support a causal relationship or to draw firm conclusions. The clinical trial setting may not be representative for the "real life" scenario (i.e. patients are older, have more comorbidities, among other factors) but the randomised, controlled nature of the clinical studies gives a robust estimate of risk in relation to placebo and other treatments. The data currently available from clinical trials do not indicate an increased risk for pancreatic cancer with these medicines. In the post-marketing setting, cases of pancreatic cancer have been reported for most products, but in a rather large number of cases there were confounding factors or, in general, too short exposure to suspect a causal relationship with the products. Again, data comparing the rate of spontaneous reporting between different products is to be interpreted with care and should always be assessed in the context of other available information (e.g. cumulative data in the periodic safety update reports and results from clinical studies).

It is noted that marketing authorisation holders are closely monitoring for effects on the pancreas. Several initiatives are planned or ongoing which will collect information on pancreatic events, and the potential value of additional studies will also be considered. In particular, cardiovascular outcome studies are ongoing for most products. For some of these studies pancreatitis and neoplasms are listed as adverse events of special interest and/or are adjudicated. The number of subjects planned to be included ranges between 6000 and 16000 patients and the studies are expected to be finalised in 2015-2017. Results from post-marketing database/registries studies with regards to pancreatic safety will also be considered when available. The data so far has been limited and does not allow conclusions to be drawn.

3. Overall conclusion

The current review under article 5(3) was initiated following the publication by *Butler at al*, 2013 suggesting that histological findings in human pancreata could indicate a possibly increased risk of pancreatic adverse events associated with the use of GLP 1 based therapies.

The CHMP reviewed the publication and considered that differences between the studied groups (diabetes with and without incretins and non-diabetic controls) with respect to age, gender, duration of diabetes and treatments as well as other methodological issues preclude meaningful interpretation of the data. This conclusion was supported by an ad-hoc expert meeting held on 10 July 2013.

Within the procedure, the CHMP was also requested to take other available data into account and a review of submitted clinical and nonclinical data was performed.

With respect to nonclinical data, available studies previously submitted for the approved products have not raised concern with respect to pancreatic safety. Further, published studies have not shown any evidence for treatment-related pancreatitis or preneoplastic lesions, neither in pancreata from healthy mice, rats and nonhuman primates nor in diabetic ZDF rat models. However, studies performed in some other disease models by academic groups may give some plausibility with respect to a possible mechanism for an increased risk of pancreatitis and pancreatic cancer in patients treated with GLP-1 based therapies.

Concerning pancreatitis, the cases in the clinical studies were few. However, when looking at the clinical studies in totality and taking post marketing reports into account, a significant number of cases have been observed and a causal relationship between GLP-1 based therapy treatment and pancreatitis is possible. Warnings are already included in the product information for all products, albeit with small differences in the wording, and pancreatitis is being followed in the periodic safety update reports as well as in observational and randomised clinical trials. These actions are considered as sufficient and no new data has emerged that implies that this risk is higher compared to what has previously been concluded. However, with the next updates of the risk management plans, pancreatitis, which should be already mentioned in the risk management plans as a potential risk should be listed as an identified risk for all products and it would be appropriate to harmonize the wording of the warning with respect to a recommendation to use the products with caution in patients with a history of pancreatitis as well as a recommendation not to resume treatment if pancreatitis has occurred.

Concerning pancreatic cancer, there is currently no support from clinical trials that GLP-1 based therapies increase the risk. The numbers of spontaneous reports are limited and in the cases were information is available, confounding factors and/or short-term exposure is common. However, long term consequences of stimulation of beta-cells and suppression of alpha cells as well as possible effects on exocrine pancreas are largely unknown and therefore some uncertainties exist. Considering that pancreatic cancers are very rare, large populations would need to be studied for a substantial duration to detect a possible increased risk. Observational studies have so far not been able to detect enough cases probably due to the rarity of the condition and, at least in Europe, rather low uptake of the products.

Additional information will be captured in the ongoing cardiovascular outcome studies. Six studies including a large number of patients are ongoing and it is expected that important information can be collected. The marketing authorisation holders should be requested to confirm that the protocols explicitly include "pancreatic malignancies/neoplasms" as an adverse event of specific interest since this might lead to increased awareness and reporting of this specific type of malignancies/neoplasms. Efforts should be made to capture pancreatic events in a similar way in the studies in order to enable a pooled analysis and consideration should be given to yearly interim reports with respect to pancreatic events (pancreatitis and pancreatic cancer). Furthermore, pancreatic cancer must be included as a potential risk for all products for which it is not already reflected in the risk management plans. Considering the low incidence of pancreatic cancer, results from the ongoing observational studies will also be of importance and therefore marketing authorisation holders should ensure that pancreatic safety is adequately captured in these studies. Other epidemiological approaches to studying this potential risk could also be considered, if appropriate.

Should new evidence indicate an increased risk of pancreatic cancer and/or a higher risk of pancreatitis compared to current estimations (e.g. from clinical studies and periodic safety update reports), the benefit-risk balance of GLP-1 based therapies should be re-evaluated. However, this should be done in a product specific manner considering that the magnitude of the benefits and risk of the products differ with respect to glucose and weight lowering capacity as well as the incidence of gastrointestinal and immunological adverse events. Furthermore, should there be an increased risk of pancreatic adverse events it is not evident that the risk is of the same magnitude for all products considering differences in mechanism of action (i.e. GLP-1 receptor agonists versus DPP-4 inhibitors) and exposure (intermittent versus continuous exposure).

In conclusion, the results of the study by *Butler et al* are not considered to constitute a new safety signal for the GLP 1 based therapies with respect to pancreatic safety. This is further supported by the review of available preclinical and clinical data.

However, due to the mechanism of action, there are still some uncertainties with respect to long term pancreatic safety associated with these products and updates to the risk management plans (including planned and ongoing studies) and harmonisation of warnings in the product information should be taken forward.

EXHIBIT 18

www.medscape.com

EU Agency Has No New Concerns on Incretin Diabetes Drugs

Lisa Nainggolan Jul 26, 2013

The European Medicines Agency (EMA) says presently available data do not confirm recent concerns over an increased risk for pancreatic adverse events with glucagon-like peptide-1 (GLP-1)–based type 2 diabetes therapies.

"There is no change in evidence regarding the risks," concludes the EMA's Committee for Medicinal Products for Human Use (CHMP), which has finalized a review of GLP-1–based diabetes therapies, also known as incretins. These comprise two classes of medicines: GLP-1 agonists and dipeptidylpeptidase-4 (DPP-4) inhibitors.

Although a slightly increased risk for pancreatitis with these products is well recognized and noted in their labeling, there is unease about the potential of a class carcinogenic effect.

Concerns in this regard arose most recently following a study, published in [illegible] in March, by Peter Butler, MD, from the David Geffen School of Medicine, University of California, Los Angeles, and colleagues. The researchers found abnormal changes, including precancerous lesions, in the pancreases of 8 organ donors taking GLP-1–based drugs.

Following the publication of Dr. Butler's study, the [illegible] published an in-depth investigation of the issue, and the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) held a 2-day meeting, at which dozens of experts discussed the topic, concluding there is currently little evidence for an increased risk for pancreatic cancer associated with use of incretins.

EMA Says Butler Study Has Limitations, Sources of Bias

The EMA says the findings of Dr. Butler and colleagues "were based on examination of a small number of pancreatic tissue samples obtained from organ donors with and without diabetes mellitus, who died due to causes other than diabetes."

Also, the study itself had many methodologic limitations and potential sources of bias, most importantly differences between the studied groups with respect to age, sex, disease duration, and treatments, "which preclude a meaningful interpretation of the results," EMA states.

In addition, data from clinical trials do not indicate an increased risk for pancreatic cancer with these medicines, it concludes.

However, EMA acknowledges that the number of events is too small to allow final conclusions. "Due to their mechanism of action (stimulation of beta-cell- and suppression of alpha cell-function) some uncertainties remain in respect to the long-term effect of these medicines on the pancreas and more data collection efforts are under way."

In the United States, too, experts say that much longer-term data will be needed to provide a definitive answer to the question of whether GLP-1–based drugs increase the risk for pancreatic cancer. Organizations including the American Diabetes Association and the Endocrine Society agree and have called on pharmaceutical companies to be transparent with their data.

In the meantime, doctors are being urged to discuss the potential adverse effects of incretin-based therapy and the symptoms of pancreatitis with patients, especially those with other risk factors for the condition, and balance the risks and benefits.

EMA Will Harmonize Warnings on Pancreatitis

EMA says a small number of cases of pancreatitis associated with these agents have been reported in clinical trials, and a significant number of cases have been recorded through adverse event reporting, "although these need to be interpreted cautiously."

All these medicines already carry pancreatitis warnings in their product information, but the agency intends to harmonize the wording of these warnings across all GLP-1–based therapies in the European Union (EU), "so that patients and healthcare professional receive consistent advice," it notes.

EMA also points out that the marketing authorization holders of these medicines are closely monitoring them for adverse effects, including effects on the pancreas, and they report their findings regularly to the agency for assessment. "Marketing authorisation holders will update the risk management plans for these medicines accordingly," it states.

GLP-1–based therapies approved in the EU include exenatide (, i d, , g zi ut ; AstraZeneca/Bristol-Myers Squibb Alliance), liraglutide (**Yrf u d**; Novo Nordisk), lixisenatide (**M s rd**; Sanofi Aventis), sitagliptin (**Bllrfi re**, **I dt rd**, **I dt s i** , **Sn dei t** , **Sn luz**, **W d i r**, **Yi rs i rd**, **ai ri rd**; Merck & Co), saxagliptin (**Lus eumr i** , **Pt mr d**; AstraZeneca/Bristol-Myers Squibb), linagliptin (**I i t dg i u**, **Wzdò t d**; Boehringer Ingelheim/Lilly), and vildagliptin (**B fzi d** , **Edr** , **Hdt gzd**, **I drzd**, **a rrdz** , **cus dzn** ; Novartis).

Ongoing and Planned Studies Will Yield More Info

EMA adds that several studies are planned, or ongoing, including large outcome studies aimed at increasing the ability to understand and quantify risks associated with these medicines, including the occurrence of pancreatitis and pancreatic cancer.

In addition, 2 large independent studies have been under way since 2011 to study the risk profile of diabetes treatments in general, and more specifically their risk profile in relation to the pancreas. First results of these studies, which are funded by the European Commission, are expected in the spring of 2014.

"In the meantime the EMA continues to closely monitor and assess all information that is becoming available on these medicines to ensure that their benefit-risk balance remains positive."

Send comments and news tips to news@medscape.net.

Cite this article: EU Agency Has No New Concerns on Incretin Diabetes Drugs. **Oi g f dyi** . Jul 26, 2013.

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EXHIBIT 19

CONFIDENTIAL TO REGULATORY AGENCIES

EXENATIDE

PERIODIC SAFETY UPDATE REPORT

01 October 2008 through 31 March 2009

(International Birth Date: 28 April 2005)

(Country of IBD: United States)

Eli Lilly and Company

Amylin Pharmaceuticals, Inc

Approved by:

Dr. Andrzej Czarnecki, MD, PhD
Director, Deputy EU Qualified Person for Pharmacovigilance
Global Patient Safety
Eli Lilly and Company Limited
Windlesham, Surrey, GU20 6PH
UNITED KINGDOM

Approval Date: **20 May 2009**

Table 47. Reporting Rates for Pancreas Cancer Among Diabetic Treatments

Treatment medication	All i3 population	i3 Patients with diagnosis of pancreas cancer	Percentage of i3 population with diagnosis of pancreas cancer
Repaglinide	12033	34	0.3
Nateglinide	12254	24	0.2
Sulfonylurea + metformin (SFU + met)	54021	77	0.1
Thiazolidinedione (TZD)	272339	338	0.1
SFU	327933	400	0.1
Insulin	263852	311	0.1
Exenatide	51452	49	0.1
Metformin	599606	552	0.1
TZD + met	72801	66	0.1

It was determined that these in-house resources did not provide an adequate number of cases for a robust analysis and would be of limited value. It was therefore determined that an outside claims database would be utilized for this study.

The purpose of this study is to utilize a claims database to characterize the incidence and prevalence of pancreas cancer (pancreatic adenocarcinoma, islet cell endocrine tumors including glucagonomas and insulinomas, and ampullary cancers arising in the ampulla of Vater) among patients with Type 2 diabetes. The primary objective is to compare the incidence of pancreas cancer among patients with Type 2 diabetes to patients without a diagnosis of diabetes Additionally, as a secondary objective, this study will also compare the incidence of pancreas cancer among patients receiving exenatide versus a matched comparison group of Type 2 diabetes patients treated with other oral antidiabetic agents and/or insulin therapies.

A feasibility assessment for the i3 contracted pharmacoepidemiologic study is ongoing. Updates will be provided when more complete i3 data is available.

9.14.8.2.5.5. Pancreas Cancer in Clinical Trial Data

There have been no new cases of pancreas cancer diagnosed in clinical trials subjects in this reporting period. Cumulatively up through this reporting period (31 March 2009), only 3 cases of pancreas cancer reported in PSUR 05 (Appendix 10, Table B) have been identified. Among them, 1 case was in the comparative (insulin) arm; the remaining 2 cases were in the exenatide arm.

9.14.8.2.5.6. Pancreas Cancer Conclusion

The global cumulative reporting rate for pancreas cancer is 4.7 cases per 100,000 PY (48 cases/1,016,420 PY) to 31 March 2009. Considering the potential for stimulated reporting due to the FDA website safety alert, under-reporting of postmarketing cases of pancreas cancer may have been reduced. The estimated incidence rate for pancreas cancers in the general adult population is in the 8-12 per 100,000 PY range (Shaib 2006). Of note, patients with diabetes have approximately a 2-fold increased risk of developing pancreas cancer compared with patients without diabetes Taking all of these data into consideration, the reporting rate for pancreas cancer in patients receiving exenatide does not appear unusual Amylin and Lilly will closely monitor the trend of pancreas cancer reports and will submit updates of the pharmacoepidemiological study as available

9.14.8.2.6. Thyroid Cancer

On 02 April 2009, an FDA advisory panel committee discussed the potential risk of medullary thyroid (c-cell) cancer based on rodent carcinogenicity studies from liraglutide (another GLP-1 analogue) with longer duration of action than the BID formulation of exenatide (BYETTA). Liraglutide caused benign and malignant thyroid c-cell tumors in rats at low multiples of human exposure and in mice at higher exposures. In contrast, the preclinical data of the BID exenatide formulation was associated with increased benign c-cell adenomas in female rats only, without any increase of tumors in mice. In the preclinical studies of the long acting, once weekly formulation of exenatide, a 2-year (life time) rat carcinogenicity study showed statistically significant more benign (both genders) and malignant (females only, at highest doses) c-cell tumors at all doses versus placebo. Also, noted was a numerical increase of malignant c-cell tumors in male rats in all doses; however, this numerical increase was not statistically significant. Because of a hypothetical GLP-1 receptor agonist effect on thyroid C-cells, a brief cumulative review was conducted for thyroid cancer in this section.

9.14.8.2.6.1. Methodology

The LSS database was searched for all spontaneous reports of thyroid neoplasms from launch to a 31 March 2009. This search was conducted using the Medical Dictionary for Regulatory Activities (MedDRA) criteria outlined in the Table 48.

EXHIBIT 20

Characterization of the Exocrine Pancreas in the Male Zucker Diabetic Fatty Rat Model of Type 2 Diabetes Mellitus Following 3 Months of Treatment with Sitagliptin

Thomas Forest, Daniel Holder, Adam Smith, Caron Cunningham, Xiaorui Yao, Markus Dey, Clay Frederick, Srinivasa Prahalada

Merck & Co., Inc., Whitehouse Station, NJ USA

Sitagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor-based incretin therapy intended for the treatment of type 2 diabetes mellitus (T2DM), has not been linked to adverse effects on the pancreas in prospective clinical trials or in nonclinical toxicology studies. To further assess potential pancreatic effects, sitagliptin was studied in the male Zucker Diabetic Fatty (ZDF) rat model of T2DM. Following 3 months of oral dosing with vehicle, or sitagliptin at doses 3- to 19-fold above the clinically therapeutic plasma concentration, which increased active plasma GLP-1 levels up to approximately 3-fold, or following 3 months of oral dosing with metformin, a non-incretin-based reference T2DM treatment, the pancreas of male ZDF rats was evaluated using qualitative and quantitative histopathology techniques. In the quantitative evaluation, proliferative index was calculated in exocrine pancreatic ducts and ductules using computer-based image analysis on sections stained by immunohistochemistry for cytokeratin (a cytoplasmic epithelial cell marker) and Ki-67 (a nuclear marker of recent cell division). Relative to controls, sitagliptin treatment did not alter disease progression based on detailed clinical signs and clinical pathology assessments. Sitagliptin treatment did not result in pancreatitis or any adverse effect on the pancreas based on a qualitative histopathology evaluation. Proliferative index did not increase with sitagliptin treatment based on quantitative assessment of more than 5000 sections of pancreas, where control group means ranged from 0.698 to 0.845% and sitagliptin-treated group means ranged from 0.679 to 0.701% (*P*=0.874). Metformin treatment was similarly evaluated and found not to have adverse effects on pancreas.

The worldwide incidence of diabetes mellitus is increasing dramatically, especially in young people and in developing countries, to the extent that 1 out of every 13 adults, or approximately 440 million individuals, are projected to have diabetes by 2030 (1, 2). During 2005–2006, two novel, incretin-based treatments for type 2 diabetes mellitus (T2DM) were introduced: glucagon-like peptide-1 (GLP-1) receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors (3). These treatments have subsequently gained wide therapeutic acceptance due to effective lowering of glycosylated hemoglobin (HbA_{1c}), low hypoglycemia risk, no effect on body weight or promoting weight loss, and the capability of being combined with metformin (3, 4). Currently marketed GLP-1 receptor agonists are variants of the endogenously produced GLP-1 peptide that are resistant to otherwise rapid degradation by DPP-4. DPP-4 inhibitors prevent degradation of endogenously produced GLP-1 as well as the related peptide, glucose-dependent insulinotropic polypeptide (GIP) (5). The increased persistence of endogenously produced GLP-1 and GIP in circulation resulting from DPP-4 inhibition is differentiated from pharmacologic administration of a GLP-1 receptor agonist, with the latter associated with an increased incidence of clinical gastrointes-

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Abbreviations:

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tinal (GI) side effects, such as nausea (3, 4). In addition to demonstrated clinical efficacy in lowering fasting and postprandial glucose levels, incretin-based therapies may have a longer term antidiabetic effect. There is preliminary evidence in rodent models of T2DM and in vitro in cultured human pancreatic β cells that suggests incretin-based therapies may slow the progressive loss of pancreatic β cells, which is characteristic of the natural progression of T2DM (6–10).

Although there are proven benefits to incretin-based treatments of T2DM, questions have been raised regarding the possibility that long-term treatment might lead to pancreatitis and potential neoplasia (8, 11–16). Similar questions have been raised about a possible link to pancreatitis for other therapeutic classes of diabetes treatments in the past (17), possibly because diabetes constitutes an independent risk factor for pancreatitis and pancreatic cancer (18–21). Recent studies evaluating large databases of insurance claims demonstrated that incretin-based therapy poses no significant increase in risk for pancreatitis over that due to diabetes alone (18, 22, 23). Long-term studies of sitagliptin, a DPP-4 inhibitor, did not demonstrate a risk of pancreatitis or neoplasia when administered to rats up to 56-fold or to mice at up to 68-fold over the human therapeutic plasma concentration, to dogs in a chronic 9-month study, or to monkeys in a 3-month study up to 28-fold above the human therapeutic plasma concentration (17). A similar lack of evidence for pancreatic risk in animal studies has been reported for GLP-1 receptor agonists (24, 25). Two prospective studies using supratherapeutic doses of currently marketed GLP-1 receptor agonists (exenatide and liraglutide) in the ZDF rat model of T2DM did not demonstrate adverse pancreatic findings (26, 27). A pilot study of exenatide in our laboratory confirmed the published absence of an adverse pancreatic effect in the ZDF rat model (data on file).

The ZDF rat (fa/fa) model of T2DM is well characterized and widely used in diabetes research because it is known to share many of the common pathophysiologic hallmarks of T2DM in humans such as obesity, insulin resistance and glucose intolerance in liver and extrahepatic tissues, progressive hyperglycemia associated with loss of pancreatic β-cell mass, and impaired carbohydrate metabolism (28). In addition, the ZDF rat develops common comorbidities of T2DM found in humans, such as lenticular cataract, retinopathy, nephrosis, neuropathy, and impaired wound healing (28). The female Zucker rat does not consistently develop hyperglycemia (28), and therefore was not considered a useful model for this study.

The beneficial effects of sitagliptin on the endocrine pancreas in diabetic rodents have previously been demonstrated (8, 9). The present study evaluated whether or not sitagliptin produced a direct or indirect adverse effect on the exocrine pancreas in a well-characterized rodent model of T2DM. Specific end points included assessments for pancreatitis, pancreatic ductal metaplasia, and proliferative rate of pancreatic duct and ductular epithelial cells. The doses used provided plasma concentrations in excess of the clinical therapeutic plasma concentration (8.5 μMhr) (29), inhibited DPP-4 activity, and increased active plasma GLP-1 levels.

Materials and Methods

Animals

A histologic evaluation was performed on 300 male ZDF rats (Table 1). Six groups of 25 rats each received vehicle and were evaluated qualitatively by light microscopy and quantitatively by computer-based morphometric methods (database controls) to provide a robust historical control ZDF database. Six groups of 25 rats each were used in the pivotal evaluation of potential effects on the pancreas. In the pivotal study, two control groups received the same vehicle used to generate the database controls; three groups received sitagliptin as a phosphate salt monohydrate suspension at 30, 100, or 150 mg/kg/d; and one group received a hydrochloride salt suspension of metformin at 450

Table 1. Group assignments for 300 male ZDF rats evaluated qualitatively by light microscopy and quantitatively by computer-based morphometric methods.

Treatment	Number of rats
Database controls	
A	25
B	25
C	25
D	25
E	25
F	25
Concurrent controls	
I	25
II	25
Sitagliptin (mg/kg/day)	
30	25
100	25
150	25
Metformin (mg/kg/day)	
450	25

Additionally, in separate arms of this study, 8 male ZDF rats were studied to determine the effect of 150 mg/kg/day of sitagliptin on total and active GLP-1 levels, and 36 male ZDF rats were studied to determine the effect of 150 mg/kg/day of sitagliptin on DPP-4 inhibition.

Exhibit 20 - 241

mg/kg/d. In addition, 44 rats were separately evaluated for pharmacologic endpoints.

The number of animals, procedures, and experimental design were in accordance with the Merck Institutional Animal Care and Use Committee. ZDF-Leprfa/Crl male rats 10 to 12 weeks of age and weighing 300 to 450 g at study start were purchased (Charles River Laboratories, Stone Ridge, NY, USA). For groups intended for histopathology evaluation (including 2 concurrent vehicle control groups and 6 vehicle control groups evaluated to establish a ZDF-Leprfa/Crl male rat database), 25 rats were prospectively assigned to each group using a randomization protocol. Rats were pair-housed in solid-bottom plastic box caging with contact bedding and provided PMI Rodent Diet® #5008, which is considered a high fat diet, (LabDiet, St. Louis, MO, USA) and tap water ad libitum. Animals were housed in environmentally controlled rooms with an approximately 12-hour light/dark cycle. For the associated studies evaluating the pharmacological effects of sitagliptin in ZDF rats, similar animal husbandry conditions were used, but the group size was 4 or 12 and the sitagliptin doses studied were 150 or 500 mg/kg/d corresponding to integrated plasma exposures (AUC) of approximately 149 and 522 μMhr.

Compound Administration

The 300 male ZDF rats evaluated histologically were dosed once daily at 5 mL/kg body weight by oral gavage based on the most recent body weight. Control rats received vehicle as 0.5% (w/v) methylcellulose with 5 mM HCl in deionized water. Sitagliptin (chemically synthesized by the study sponsor, Merck & Co., Inc., Whitehouse Station, NJ, USA) was administered as a suspension in 0.5% (w/v) methylcellulose with 5 mM HCl in deionized water. Doses of 30, 100, and 150 mg/kg/d of sitagliptin were chosen based on data from previous studies suggesting that these doses would produce, respectively, 3, 10, and 17 times the human clinically efficacious exposure ($AUC_{0-24\ hours}$) of 8.5 μMhr (29). Metformin (Framhispania, S.A., Spain) was administered as a suspension in 0.5% (w/v) methylcellulose in deionized water at 450 mg/kg/d based on the estimated maximum tolerated dose in male rats in the most recent marketing approval for metformin and targeting approximately 8-fold the human clinically efficacious exposure (30–32). Samples of sitagliptin and metformin dosing formulations were assayed for concentration and uniformity in Week 1 and for concentration in Week 12. All assay results were within the acceptable range (±15% of claim for a suspension) for concentration and uniformity. The same dosing procedures were used to evaluate the pharmacologic effects of sitagliptin in ZDF rats.

Clinical observations

For rats intended for histologic endpoints, body weight and food consumption data were collected once weekly. Physical sign observations were performed daily. Approximately 2 mL of blood was collected via the retro-orbital route under isoflurane anesthesia during Weeks 4 and 12 for hematology and blood chemistry analysis. During Week 12, an overnight urine collection was performed to measure volume, pH, and specific gravity. Prior to initiation of dosing and in Weeks 2, 8, and 13, a non-fasted whole blood sample was collected from the tail vein of each animal between 7:00 and 9:00 am before dosing to measure glucose level via a glucometer (LifeScan OneTouch Ultra Glucometer, Milpitas, CA, USA). In Week 12, plasma (approximately 350 μL per sample) was collected by tail vein for determination of circulating levels of sitagliptin or metformin at nominal times of 0.5, 1, 2, 4, 8, and 24 hours post dose from concurrent control and treated rats. Due to blood volume sampling limitations from individual rats, samples for drug levels were collected using a scheme that used a subgroup of rats at each time point, but resulted in an equal total volume collected from each rat over the time course. The assays for sitagliptin and metformin were conducted by a validated bioanalytical method (compliant with regulatory quality standards) (33) using liquid chromatography/tandem mass spectrometry. The 1-hour plasma samples were assayed from the two concurrent control groups and found not to have test article contamination.

Plasma DPP-4 inhibition (Merck & Co., Inc.) and total and active plasma GLP-1 levels (Meso Scale Discovery, Rockville, MD, USA) were measured in male ZDF rats intended for pharmacology end points in a group treated with sitagliptin at 150 mg/kg/d from samples collected at 0.5, 1, 2, 4, 8, and 24 hours post dose using a collection scheme similar to that described above for DPP-4 inhibition, or at 0, 2, 4, 8, 12, 16, and 20 hours after a single dose for total and active GLP-1 levels.

Pathology evaluation

At study end, the rats were fasted overnight, weighed, anesthetized with isoflurane, and euthanized by caval exsanguination. A complete necropsy was performed, and pancreas and brain weights were recorded. For organ weight comparison, a trend analysis with multiplicity adjustment was used between sitagliptin-treated rats and concurrent controls. The entire pancreas from all rats was fixed at room temperature in 10% neutral buffered formalin for 36 hours ± 4 hours.

To ensure that each rat in a given treatment group made a balanced contribution to the total pancreatic area evaluated, nine segments, representative of the entire pancreas from head-to-tail, were collected from all rats. Trimming was done using a systematic random sampling scheme to allow an unbiased and complete assessment of each pancreas. The nine tissue segments to be evaluated from each rat were embedded in three paraffin blocks (3 segments per block), so that each block included a segment from the head, body, and tail of the pancreas.

An approximately 5-μm paraffin section of pancreas was prepared from the three blocks from each rat and stained with hematoxylin and eosin (H&E). A second approximately 5-μm adjacent section was prepared for immunohistochemical staining. In all cases, paraffin sectioning of blocks (microtomy) was performed the day before initiating the immunohistochemistry staining process, so that the interval between microtomy and immunohistochemistry staining was similar for all slides.

The qualitative light microscopic evaluation conformed to Good Laboratory Practice Standard Operating Procedure methods, which are fully compliant with the best practices outlined by the Society of Toxicologic Pathology (Crissman et al 2004), and are the standard practice for generating nonclinical data submissions to support drug registration, but do not involve blinding the pathologist to treatment group.

Cell proliferation evaluation

Proliferative index (PI) in pancreatic duct and ductular epithelial cells in the exocrine pancreas was determined for 300 male

ZDF rats. To minimize lot-to-lot variability in reagents for concurrent control groups I and II and treated rats, supplies for immunohistochemical staining were purchased in bulk before study start. For all rats studied, slide deparaffinization and antigen retrieval were conducted in a single processing step in a module (Thermo Scientific Pretreatment Module, Waltham, MA, USA) with precision temperature controls, and with sufficient capacity to hold all the slides from one staining batch. A single immunohistochemistry stainer (DAKOCytomation Autostainer Plus, Carpentaria, CA, USA) was used. To limit the impact of variability in immunohistochemical staining, all batch processing steps were conducted so that each dose group was equally represented in each processing batch. The immunohistochemical approach used was a dual-staining method using pancytokeratin (1:30,000; Abcam ab6401, Cambridge, MA, USA) and Ki-67 (1:1600; Abcam ab16667) antibodies. The pancytokeratin antibody preferentially stained the cytoplasm of epithelial cells in pancreatic ducts and ductules. The Ki-67 antibody stained the nuclei of recently mitotically active cells.

All nine pancreatic sections stained immunohistochemically from each scheduled sacrifice rat were digitally scanned with a digital whole slide scanner (Aperio ScanScope XT, Vista, CA, USA) using a 20× objective and annotated (Aperio ImageScope). Analysis for PI was conducted on the entire section of pancreatic parenchyma from each of the nine sections from each rat by the Cytonuclear Tool (Indica Labs, Corrales, NM, USA) computer image analysis algorithm. Appropriate settings for the Cytonuclear Tool algorithm were determined in pilot experiments, and the settings were established prior to analysis. All PI measurements reported herein were made using identical algorithm settings using all the pancreatic tissue on the slides. Duct and ductular epithelial cells were identified by the presence of sufficient cytokeratin staining within a specified distance from the nucleus. Proliferative index was determined by dividing the number of recently mitotic epithelial cells (cells positive for pancytokeratin and positive for Ki-67) by the total number of epithelial duct and ductular cells (cells positive for pancytokeratin), and multiplying the resulting value by 100.

For rats evaluated at scheduled study termination, the nine sections stained with H&E and the nine adjacent sections stained immunohistochemically were evaluated qualitatively by a pathologist, and a peer review of all of these slides was conducted by a second pathologist. In the case of rats found dead during the study, pancreas sections were prepared and stained with H&E only using the same methods as for the scheduled sacrifice rats. These rats were not included in the quantitative evaluation because pancreas tissue from these animals was not suitable for immunohistochemical staining within the quality specifications required for a suitably accurate measurement of proliferative index.

Statistical analysis

For hematology and serum biochemistry parameters, tests for normality (Wilk-Shapiro statistic) and homogeneity of variance (Levene's test) were conducted on each parameter for each time interval. The Dunnett's multiple comparisons test was conducted to determine statistically significant differences ($P \leq .05$) between individual treatment group and the concurrent control group I means. Mean active and total GLP-1 AUC were compared between control and treatment groups by Student's t test ($P \leq .05$).

Per the prespecified statistical plan, the significance of trends in pancreatic weight in sitagliptin-treated rats (ie, an increase or decrease with increasing dose of sitagliptin) was assessed by comparing absolute weight, percentage of body weight, and percentage of brain weight relative to control group I. P-values were reported with adjustment (Dunnett's) for multiplicity of tests. Statistical significance was set at $P \leq .05$.

For PI in the exocrine pancreas, one-way analysis of variance (ANOVA) model (ANOVA) was fit to the angular transformation (arcsine square root) of proliferative index, with the groups determined by the treatment regimen administered. The null hypothesis that the PI does not increase with dose was tested against the alternative of an increasing dose trend using a linear contrast. This contrast effectively averages the two control groups.

Results

Body weights, food consumption, hematology, and clinical chemistry

There were no differences noted in body weight, food consumption, complete blood count (CBC), or urinalysis parameters in sitagliptin-treated rats. Fasting plasma glucose after 12 weeks (**Supplemental Table 1**) and nonfasting blood glucose (Figure 1) were consistent with diabetes mellitus, and not significantly changed by treatment with sitagliptin. The slight decrease in fasting plasma glucose in Week 4 in the group treated with sitagliptin 150 mg/kg/d vs the control group was not statistically significant ($P >$ .05) (Supplemental Table 1). Anticipated age-dependent increases in fasting glucose in the ZDF model were observed.

Decreases in triglycerides observed in sitagliptin-treated groups Weeks 4 and 12 were statistically significant ($P \leq .05$) at Week 4 for sitagliptin 100 mg/kg/d (6.73 mmol/L [596 mg/dL]) relative to the control I (9.35 mmol/L [827 mg/dL]) and at Week 12 for sitagliptin 150 mg/kg/d (8.17 mmol/L [723 mg/dL]) relative to the control

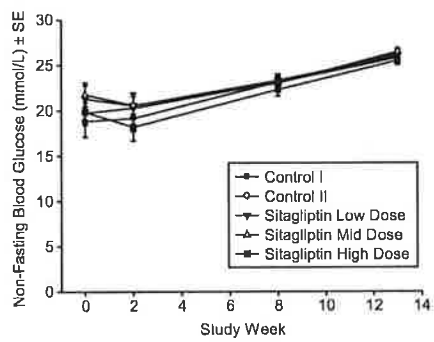

Figure 1. Nonfasting blood glucose (mean ± standard error) in control I and II and sitagliptin-treated male Zucker diabetic fatty rats. Sitagliptin low dose = 30 mg/kg/d; sitagliptin mid dose = 100 mg/kg/d; sitagliptin high dose = 150 mg/kg/d.

I (10.17 mmol/L [900 mg/dL]). The general similarity between control and sitagliptin groups in body weight change, food consumption, and in blood chemistry measures suggests little difference in manifestation of T2DM in the ZDF rat model due to sitagliptin-treatment. In-life study findings in 150 database control ZDF rats (Table 1) evaluated in preparation for the sitagliptin study were similar to those of the 50 ZDF rats in concurrent control groups I and II.

Sitagliptin plasma exposure kinetics and pharmacology

The integrated sitagliptin plasma concentrations were 2.7×, 12×, and 19× the therapeutic clinical exposure ($AUC_{0-24\ hours}$) of 8.5 μMhr (29) at doses of 30, 100, and 150 mg/kg/d, respectively, as summarized in Supplemental Table 2. Sitagliptin pharmacokinetics in Week 12 demonstrated adequate exposure margins relative to the therapeutic clinical plasma exposure.

In a separate group of male ZDF rats studied for pharmacology end points were treated for 12 weeks with sitagliptin 150 mg/kg/d (plasma AUC_{0-24hr} of 149 ± 9.26 μMhr in Week 12), DPP-4 inhibition was greater than 90% from 0.5 to 8 hours post dose, and greater than 80% at 24 hours post dose. Therefore, the integrated sitagliptin plasma concentrations achieved in the 150 mg/kg/d group in this study significantly exceeded the human therapeutic plasma concentrations and were sufficient to test the possibility that DPP-4 inhibition might affect the pancreas in this model.

After a single dose of sitagliptin at approximately 150 mg/kg/d (n = 4/group), levels of total GLP-1 were unchanged (P = .590), but levels of active GLP-1 were increased approximately 3-fold (P = .003). Plasma glucose levels were not significantly affected by treatment (P = .439) (**Figure 2**).

In-life findings with metformin treatment and plasma exposure kinetics

In the group treated with metformin at 450 mg/kg/d (plasma AUC_{0-24hr} of 1430 ± 102 μMhr in Week 12) there was an increase in mean body weight (+86 g) compared to controls (+44 g) over the course of the study, approximately 20% less weekly food consumption than controls, and approximately 40% greater increase in triglyceride levels compared to controls. At the end of the study, the fasted blood glucose in the metformin group was decreased (14.21 mmol/L [256 mg/dL]) compared with control group I (18.87 mmol/L [340 mg/dL]) ($P \leq .05$) consistent with less progression of disease in this group compared to other groups. The metformin AUC_{0-24hr} of 1430 ± 102 μMhr provided an approximately 8-fold multiple of the AUC_{0-24hr} at the maximum recommended clinically therapeutic dose of 2000 mg/d.

Mortality, pancreas weight, gross changes, and histology findings

Mortality was equally distributed across treatments (1 concurrent control, 1 sitagliptin-treated, 1 metformin-treated). In our laboratory many cases of spontaneous mortality in male ZDF rats are associated with apparent obstruction of the urinary tract characterized grossly by

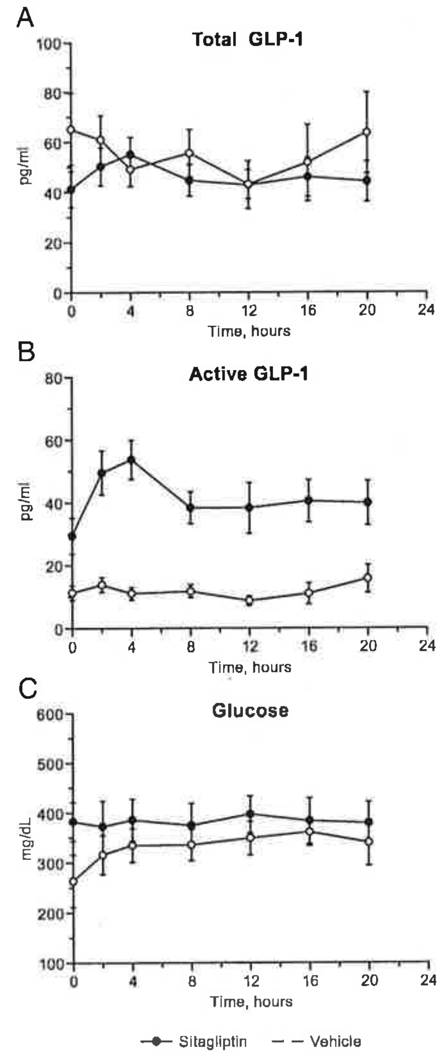

Figure 2. Total and active GLP-1 levels and plasma glucose following a single dose of sitagliptin. (150 mg/kg/d), total GLP-1 was unchanged (P = .590), active GLP-1 was increased approximately 3-fold (P = .003) based on Student's t test of AUC, and plasma glucose was unchanged (P = .439). Conversion from metric to SI units: active GLP-1: picograms/mL × 0.3032 = picomoles/L; total GLP-1: picograms/mL × 0.2432 = picomoles/L; glucose: mg/dL × 0.0555 = mmol/L.

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various combinations of dilation of the renal pelvis in kidney, distension of the ureters, dilation thickening and red discoloration of the bladder, swelling of the prepuce, and red discoloration of the penis. The three rats that died spontaneously on this study had combinations of such gross changes in the urinary tract. There were no sitagliptin-related changes in pancreas weight as absolute weight, a percentage of body weight, or a percentage of brain weight (Supplemental Table 3). Based on gross examination and qualitative light microscopic evaluation, there were no treatment-(sitagliptin) related findings in the pancreas and no evidence of pancreatitis in any sitagliptin-treated rat. The incidence of exocrine pancreatic lobular atrophy and exocrine pancreatic acinar hyperplasia, two focal background pancreatic changes that exhibited a qualitative increase in Ki-67 staining, are summarized in Table 2. The incidence of lobular atrophy and acinar hyperplasia in groups of sitagliptin-treated rats was less than the highest incidence observed in control database groups (A-F) and concurrent control groups (I and II).

Table 2. Incidence of focal background findings in exocrine pancreas ($n = 25$ per group)

	Control database						Concurrent control		Sitagliptin (mg/kg/day)		
	A	B	C	D	E	F	I	II	30	100	150
Exocrine pancreas											
Lobular Atrophy	0	1	1	3	2	3	2	1	2	0	2
Acinar Hyperplasia	3	4	8	4	9	3	3	7	4	2	4

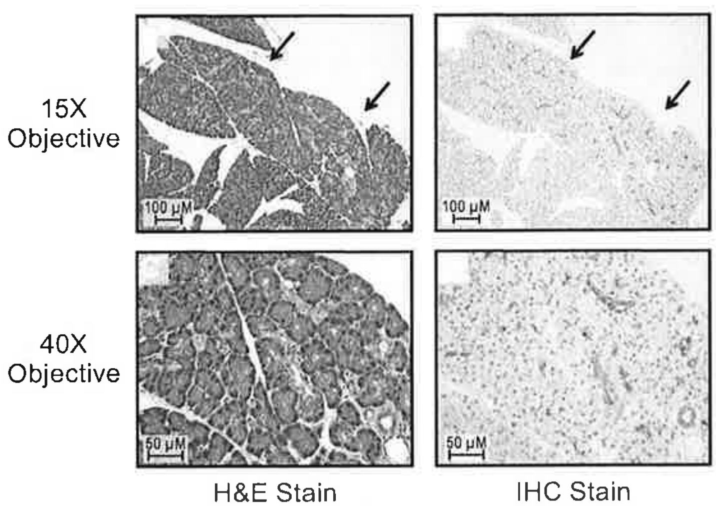

Figure 3. A representative example of pancreatic lobular atrophy, a common spontaneous degenerative finding in laboratory rats (H & E; Panel A scale bar = 100 μM; Panel B scale bar = 50 μM) that exhibits qualitatively increased staining for Ki-67 in serial sections (IHC+hematoxylin; Panel C scale bar = 100 μM; Panel D scale bar = 50 μM). Note that Ki-67 is most commonly expressed in small duct and ductule cells in foci of pancreatic lobular atrophy.

The exocrine pancreatic lobular atrophy observed as a background finding not related to treatment was an isolated focal change characterized by a localized decrease in number of exocrine acinar epithelial cells delineated by the margins of an individual pancreatic lobule (**Figure 3**, Panel A and B). Affected lobules commonly had relatively prominent ductular profiles and a relatively increased number of nuclei positive for Ki-67 compared to unaffected lobules (**Figure 3**, Panel C and D). The pancreatic parenchyma affected by lobular atrophy comprised a small portion of the total cross-sectional area evaluated, and was qualitatively indistinguishable from the small foci of lobular atrophy that are routinely identified during light microscopic evaluation in strains of laboratory rat typically used in nonclinical studies of a similar duration.

The exocrine pancreatic acinar hyperplasia observed as a background finding not related to treatment was an isolated focal background change characterized by a circumscribed collection of acinar epithelial cells arrayed in a glandular to tubulo-glandular pattern (**Figure 4**). In foci of acinar hyperplasia, there were more nuclei positive for Ki-67 compared to unaffected parenchyma (**Figure 4**). Similar to lobular atrophy, acinar hyperplasia is routinely observed as a spontaneous change in laboratory rats typically used in nonclinical studies.

The incidence and severity of exocrine pancreatic lobular atrophy and acinar hyperplasia were comparable among control database groups, concurrent control groups, and treated groups (Table 2). These focal areas of change were included in the quantitative evaluation for calculation of proliferative index.

In metformin-treated rats there was an approximately 20% increase in mean pancreas weight compared to concurrent control group I when evaluated based on absolute weight or when normalized to brain weight ($P \leq .05$), but no relevant difference when normalized to body weight (3.7% increase). Mean terminal body weight was increased in metformin-treated rats, and therefore the increase in pancreas weight on an absolute basis or normalized to brain weight was considered to be an adaptive response to the increase in body weight. There were no metformin-related gross or qualitative light microscopic findings in the pancreas, and a similar incidence of lobular atrophy (2 per 25 rats) and acinar hyperplasia (6 per 25 rats) to control rats.

Quantitative histopathology evaluation of the exocrine pancreas

In all rats surviving to study end, PI was measured by means of a commercially available computer algorithm (Cytonuclear Tool, Indica Labs) applied to all of the pancreas tissue present on each slide and evaluated using algorithm settings established before the evaluation by optimization on slides from pilot studies. Use of predetermined algorithm settings and an automated data collection process encompassing the entire section avoided the introduction of observer bias into the data collection or the need to make estimates of PI based on subsamples. The algorithm identified nuclei based on size, shape, and intensity of hematoxylin staining (**Figure 5**, Panel A and B). Ki-67–positive nuclei were identified by the presence of DAB staining. Duct and ductular epithelial cells were identified by the presence of sufficient cytokeratin staining within a specified distance from the nucleus.

Visual inspection of the distribution of PI across the six control database groups, two concurrent control groups, and sitagliptin-treated groups (**Figure 6**, Panel A and B; and **Supplemental Table 4**) confirms the uniformity of variability between groups as well as the similarity of means and medians. Mean and median PI for sitagliptin-treated groups were less than concurrent control group I. There was no increasing sitagliptin dose trend for PI ($P = .874$).

The mean ± SD PI for the metformin-treated group was 0.873 ± 0.295. Comparison between the metformin and control groups was not part of the prospective statistical plan, and meaningful comparison between the metformin group and other groups was complicated by metformin treatment-related differences in disease progression in this model, illustrated by differences in blood glucose changes, terminal body weight, food consumption, and pancreas weight (described above).

Discussion

In the ZDF rat model of T2DM, sitagliptin did not adversely affect the pancreas across a range of integrated plasma concentrations that inhibited DPP-4 activity and exceeded the human therapeutic plasma concentration target by 3- to 19-fold. As anticipated, sitagliptin had no effect on total GLP-1 levels, but increased active GLP-1 levels approximately 3-fold. Both potential pharmacologic and toxicologic effects were evaluated, and there was no evidence of pancreatitis and no evidence of an increase in PI. The assessments in this study were adequately controlled, complete, unbiased, and sufficiently powered (N = 25 per group at study start with 2 concurrent control groups and 6 control database groups) to detect small treatment-related effects. A range of sitagliptin doses were evaluated to explore the possibility of an inhibitory toxicologic effect of sitagliptin at high exposures on any of the measured endpoints and to increase the possibility of detecting a dose-dependent toxicity by a dose trend analysis.

Although the anticipated pharmacologic effects of sitagliptin were observed in this study (DPP-4 inhibition and active GLP-1 increase), there was little effect of the observed pharmacology on progression of T2DM, likely due to the fact that diabetes in the ZDF rat is driven by insulin resistance (28). Consistent with this idea, metformin, an insulin sensitizer, did impact progression of T2DM in this study (discussed below). The observed lack of a notable effect of sitagliptin-pharmacology on disease progression was a desired feature of the study design, because it

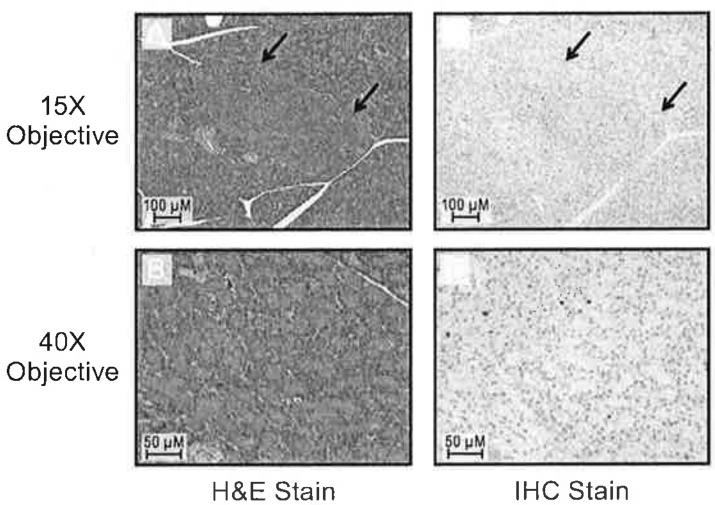

Figure 4. A representative example of pancreatic acinar hyperplasia, a spontaneous background finding with an increased incidence in laboratory rats fed a high-fat diet (H & E; Panel A scale bar = 100 μM; Panel B scale bar = 50 μM) that exhibits qualitatively increased staining for Ki-67 in serial sections (IHC+hematoxylin; Panel C scale bar = 100 μM; Panel D scale bar = 50 μM). Note that Ki-67 is most commonly expressed in pancreatic acinar cells in foci of acinar hyperplasia.

allowed direct comparison between control and sitagliptin-treated groups with similar T2DM disease burdens. If sitagliptin-treatment induced pharmacology had notably altered progression of T2DM, then it would have been difficult to separate the beneficial secondary effects of sitagliptin-pharmacology in the model from any observed toxicological effects. Similar T2DM disease burdens between control and sitagliptin-treated groups allowed an evaluation for potential adverse sitagliptin effects in the face of hyperglycemia and T2DM comorbidities. The lack of adverse sitagliptin-effects in rodents without a specific disease predilection has been documented elsewhere (17).

The ZDF rat model of T2DM that was used in this study has been widely studied by others, is well characterized, and shares many of the common pathophysiologic hallmarks of T2DM in humans. The absence of a detectable adverse effect with a DPP-4 inhibitor in this study is consistent with findings recently reported from nonclinical studies of GLP-1 agonists in ZDF rats (26, 27). Literature reports of a lack of adverse findings and no increase in PI in the pancreas in the ZDF rat model treated with exenatide were confirmed in a small pilot study (N = 12 per group) in our laboratory (unpublished results), where up to 250 μg/kg/d of exenatide was administered for 3 months and mean fasting blood glucose in Week 8 was 14.04 mmol/L (253 mg/dL) compared with a mean control value of 17.04 mmol/L (307 mg/dL). The lack of adverse changes in the pancreas in prospective studies in ZDF rats is consistent with results in nonclinical toxicology studies of DPP-4 inhibitors and GLP-1 agonists with nondiabetic animals (17, 24, 34) and is consistent with results from well-controlled epidemiologic studies of humans (18, 22, 23).

In the course of conducting the qualitative evaluation, two types of spontaneous focal background changes (exocrine pancreatic lobular atrophy and acinar hyperplasia) were identified in the exocrine pancreas with a qualitatively increased staining of nuclei for Ki-67 compared to unaffected exocrine parenchyma. Lobular atrophy, like that observed in this study, is recognized as a spontaneous change in laboratory rats used in nonclinical safety assessment studies, and is commonly attributed to obstruction of a small duct draining the affected lobule (35). The increased staining of nuclei with Ki-67 in areas of lobular atrophy may reflect activation of a program of tissue repair in response to injury.

Similarly, focal acinar hyperplasia is a spontaneous change that is routinely observed in strains of laboratory rats typically used in nonclinical studies (35). However, the incidence of acinar hyperplasia is increased in aged rats, and an increased incidence is also reported in younger rats in association with feeding high-fat diets (36), such as the diet used in this experiment. Therefore, while the incidence of acinar hyperplasia in the high fat diet ZDF rat model is higher than for some strains and feeding regimens used to conduct nonclinical safety assessment studies, the increased incidence that was observed

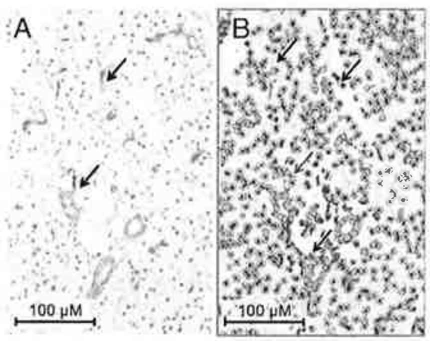

Figure 5. A representative field of exocrine pancreas (IHC+hematoxylin; 20× objective) with ducts and ductules indicated with arrows (Panel A). Same field with computer annotation used to measure proliferative index (Panel B). Black circles describe the area evaluated for cytokeratin staining to assign either a cytokeratin positive or negative status to nuclei. Arrows denote dark blue nuclei, which were Ki-67-/cytokeratin-; red nuclei were Ki-67+/cytokeratin-; green nuclei were Ki-67-/cytokeratin+; and light blue nuclei were Ki-67+/cytokeratin+; scale bar = 100 μM)

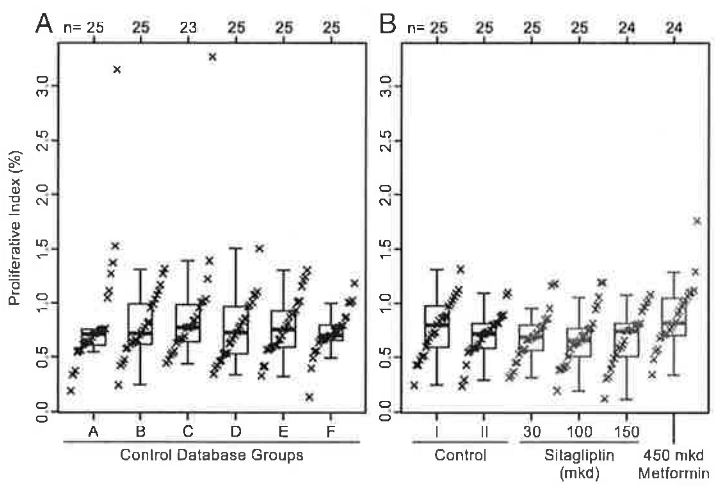

Figure 6. Distribution of proliferative index measures for individual control database rats (Panel A) and concurrent controls and sitagliptin-treated rats (Panel B) (horizontal bar = group mean) with no increasing sitagliptin dose trend ($P = .874$).

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was anticipated (37). In the rat, acinar hyperplasia has been suggested to be part of a continuum of change that includes exocrine pancreatic neoplasia, which has been observed with increased incidence in rats fed high fat diets or gavaged with corn oil vehicle in carcinogenicity studies (36, 38). Therefore, given the well-established links between the incidence of neoplasia in the laboratory rat and type of diet and food consumption (37), it is necessary to control for diet, food consumption, and body weight in studies with a prospective intent to assess cancer risk.

Because acinar hyperplasia in the rat has been suggested to be part of a continuum of change that includes exocrine pancreatic neoplasia, any treatment-related increased incidence of this finding might be evaluated as a potential risk factor for the exocrine pancreas. The lack of difference in acinar hyperplasia between control and treated groups in this study suggests that sitagliptin and metformin do not promote the development of exocrine pancreatic tumors in the rat. Due the lack of statistical power inherent in comparisons of low incidence events such as the observed rates for acinar hyperplasia and lobular atrophy in this study, formal testing of proliferative index between foci from control and treated groups was not attempted.

The absence of an increase in proliferative index in the sitagliptin treatment groups in this study is also evidence against the hypothesis that sitagliptin increases the risk of exocrine pancreatic neoplasia by increasing proliferative index. However, this method of assessing increased risk has not been validated (39). The validated, widely-used, traditional rodent bioassay is a better established and understood tool for making risk assessments of chemical exposure for human carcinogenic risk. Notably, the mouse and rat bioassays conducted for sitagliptin did not suggest a risk for pancreatic cancer (17).

The absence of pancreatitis in rats treated with sitagliptin in this study with the ZDF rat model of T2DM is consistent with results from previously reported large well-controlled toxicology studies in rodents (17), but differs from reported results in a smaller study of sitagliptin with the HIP rat model of T2DM, where 6 to 8 rats per group were studied and pancreatitis was observed post hoc in one rat treated with sitagliptin (8). In the HIP rat experiment with sitagliptin, the primary study objective was to evaluate structural and functional effects on the endocrine pancreas, and as part of a functional evaluation of the pancreatic islets, an intravenous (IV) bolus of arginine was administered following a lengthy IV administration of glucose as part of a glucose clamp assessment (8). Intraperitoneal dosing of arginine in the rat is widely used as an experimental model of induced pancreatitis (40), but a dose response for induction of pancreatitis in the HIP rat following bolus IV dosing of arginine has not been reported, making it challenging to interpret the reported pancreatic findings.

In this study, treatment with metformin meaningfully modified the progression of disease in the ZDF rat model (less severe blood glucose changes, increased absolute pancreatic weight, increased body weight, decreased food intake). Accounting for pharmacologically-induced changes in disease progression is necessary to make toxicologically relevant comparisons between the metformin group and other groups. In the absence of a control group with an equivalent disease burden, such comparisons were not attempted for the metformin group. In this study, the similarity of the qualitative changes between the metformin group and control groups is consistent with an absence of toxicologically relevant adverse effects on the pancreas, which is also consistent with the historical clinical experience with metformin.

In summary, sitagliptin had no adverse effect on the pancreas of the ZDF rat model of T2DM. There was no evidence of increased risk of pancreatitis, and no difference in exocrine duct and ductular proliferative index between treated and untreated rats.

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Address all correspondence and requests for reprints to: Thomas Forest, Merck & Co. Inc., WP45-223, 770 Sumneytown Pike, West Point, PA 19486, Email: thomas_forest@merck.com, Phone: 215-652-1575

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